Application of Artificial Neural Networks for Understanding and Diagnosing the State of Mastitis in Dairy Cattle

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K.J. Hassan

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Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Master of Applied Science.

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Bovine mastitis adversely affects the dairy industry around the world. This disease is caused by a diverse range of bacteria, broadly categorised as minor and major pathogens. In-line tools that help identify these bacterial groupings in the early stages of the disease are advantageous as timely decisions could be made before the cow develops any clinical symptoms. The first objective of this research was to identify the most informative milk parameters for the detection of minor and major bacterial pathogens. The second objective of this research was to evaluate the potential of supervised and unsupervised neural network learning paradigms for the detection of minor infected and major infected quarters in the early stages of the disease. The third objective was to evaluate the effects of different proportions of infected to non-infected cases in the training data set on the correct classification rate of the supervised neural network models as there are proportionately more non-infected cases in a herd than infected cases.

A database developed at Lincoln University was used to achieve the research objectives. Starting at calving, quarter milk samples were collected weekly from 112 cows for a period of fourteen weeks, resulting in 4852 samples

with complete records for somatic cell count (SCC), electrical resistance, protein percentage, fat percentage, and bacteriological status. To account for the effects of the stage of lactation on milk parameters with respect to days in milking, data was divided into three days in milk ranges. In addition, cow variation was accounted for by the sire family from which the cow originated and the lactation number of each cow.

Data was pre-processed before the application of advanced analytical techniques. Somatic cell score (SCS) and electrical resistance index were derived from somatic cell count and electrical resistance, respectively. After pre-processing, the data was divided into training and validation sets for the unsupervised neural network modelling experiment and, for the supervised neural network modelling experiments, the data was divided into training, calibration and validation sets.

Prior to any modelling experiments, the data was analysed using statistical and multivariate visualisation techniques. Correlations ($p<0.05$) were found between the infection status of a quarter and its somatic cell score (SCS, 0.86), electrical resistance index (ERI, -0.59) and protein percentage (PP, 0.33). The multivariate parallel visualisation analysis validated the correlation analysis. Due to significant multicolinearity [Correlations: SCS and ERI (-0.65: $p<0.05$); SCS and PP (0.32: $p<0.05$); ERI and PP (-0.35: $p<0.05$)], the original variables were decorrelated using principle component analysis. SCS and ERI were found to be the most informative variables for discriminating between non-infected, minor infected and major infected cases.

Unsupervised neural network (USNN) model was trained using the training data set which was extracted from the database, containing approximately equal number of randomly selected records for each bacteriological status [not infected (NI), infected with a major pathogen (MJI) and infected with a minor pathogen (MNI)]. The USNN model was validated

with the remaining data using the four principle components, days in milk (DIM), lactation number (LN), sire number, and bacteriological status (BS). The specificity of the USNN model in correctly identifying non infected cases was 97%. Sensitivities for correctly detecting minor and major infections were 89% and 80%, respectively.

The supervised neural network (SNN) models were trained, calibrated and validated with several sets of training, calibration and validation data, which were randomly extracted from the database in such a way that each set has a different proportion of infected to non-infected cases ranging from 1:1 to 1:10. The overall accuracy of these models based on validation data sets gradually increased with increase in the number of non-infected cases in the data sets (80% for the 1:1, 84% for 1:2, 86% for 1:4 and 93% for 1:10). Specificities of the best models for correctly recognising non-infected cases for the four data sets were 82% for 1:1, 91% for 1:2, 94% for 1:4 and 98% for 1:10. Sensitivities for correctly recognising minor infected cases for the four data sets were 86% for 1:1, 76% for 1:2, 71% for 1:4 and 44% for 1:10. Sensitivities for correctly recognising major infected cases for the four data sets were 20% for 1:1, 20% for 1:2, 30% for 1:4 and 40% for 1:10. Overall, sensitivity for the minor infected cases decreased while that of major infected cases increased with increase in the number non-infected cases in the training data set. Due to the very low prevalence of MJI category in this particular herd, results for this category may be inconclusive.

This research suggests that somatic cell score and electrical resistance index of milk were the most effective variables for detecting the infection status of a quarter followed by milk protein and fat percentage.

The neural network models were able to differentiate milk containing minor and major bacterial pathogens based on milk parameters associated with mastitis. It is concluded that the neural network models can be developed and

incorporated into milking machines to provide an efficient and effective method for the diagnosis of mastitis.

Keywords: Mastitis; Minor and Major Bacterial Pathogens; Somatic Cell Count; Electrical Resistance; Principle Component Analysis; Unsupervised Neural Networks, Supervised Neural Networks.

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It was a great experience.

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Glossary of Abbreviations

ANN	Artificial Neural Networks
ANOVA	Analysis of Variance
BS	Bacteriological status
CCR	Correct Classification Rate
CI	Conductivity Index
CM	Clinical Mastitis
CMI	Composite Milk Index
CMT	California Mastitis Test
DIM	Days in Milk
EC	Electrical Conductivity
FP	Fat Percentage
ER	Electrical Resistance
ERI	Electrical Resistance Index
IMI	Intra-mammary Infection
IQR	Inter-Quarter Ratio
LN	Lactation Number
LRM	Logistic Regression Model
MAA	Milk Amyloid A
MJI	Major Pathogen Infection
MLP	Multi Layer Perceptron
MNI	Minor Pathogen Infection
MPVA	Multivariate Parallel Visualization Analyses
NI	No Infection
NMC	National Mastitis Council
NN	Artificial Neural Networks
NS	Neighbour Strength
PCA	Principle Component Analysis
PP	Protein Percentage
SCC	Somatic Cell Count
SCM	Sub-clinical Mastitis
SCS	Somatic Cell Score
SN	Sire Number
SNN	Supervised Neural Network
SOFM	Self Organizing Feature Map

SOM	Self Organising Map
USNN	Unsupervised Neural Network
WMT	Wisconsin Mastitis Test

CHAPTER 1

1. INTRODUCTION

Mastitis is a very complex and a costly disease for the dairy industry around the world and is associated with economic losses due to decreased milk quality, milk production, and increased veterinary and labor costs (Holmes et al., 2002; Losinger, 2005; Miller, Bartlett, Lance, Anderson, & Heider, 1993; Mungube et al., 2005; Seegers, Fourichon, & Beaudeau, 2003). Mastitis is also one of the reasons for early culling decisions (Grohn et al., 2005).

In the USA, 70% of the total losses on a dairy farm are associated with mastitis and the cost per cow per year is around US$200 (NMC, 2006). In the United Kingdom, the prevalence of mastitis is approximately one million cases per year, costing 100M-400M sterling pounds to dairy farms (IAHUK, 2003). The monetary loss due to mastitis for the New Zealand dairy industry is around NZ$ 180 million per year (NMAC, 2006).

Bovine mastitis is an inflammatory response of the mammary gland to pathogenic bacterial microorganisms. These organisms are of two main types, major and minor (Harmon, 1994) Major pathogens cause bigger changes in milk parameters compared to minor pathogens. The most common major pathogens are *Staphylococcus aureus* (SA), *Streptococcus uberis* (SU), *Streptococcus agalactiae* (SA), *Streptococcus dysgalactiae* (SD), *Escherichia coli* (EC) and *Klebsiella spp.* (KS), while some common minor pathogens are *Coagulase-negative staphylococci* (CNS), *Corynebacterium bovis* (CB) and *Arcanobacterium pyogenes* (AP) (Brown, 1976; Harmon, 1994).

Bovine mastitis may be either clinical (CM) (with visible symptoms like fever, depression, inflammation, abnormal milk, loss of weight, shivering, and loss of appetite) or sub-clinical (SCM) (no visible symptoms as in CM) depending on the stage of the disease, virulence of the pathogen, immune

response of the host and the resulting symptoms (Livestock-Improvement, 2001; Schalm, Carroll, & Jain, 1971; Sharif et al., 1998).

Major pathogens are generally the cause of CM, observable by the milker and characterised by obvious physical changes in milk appearance, such as clots, blood or flakes. In some cases, minor pathogens are also able to cause CM. Detection of intra-mammary infection (IMI) in the early stages of infection would be advantageous as timely management decisions could be made before CM occurs.

1.1 Research Aim

The central aim of this research is to contribute to a better understanding of bovine mastitis by studying the milk parameters during the progression of the disease. Based on this main aim, the following specific objectives were set:

1. Identify the most informative biological and physical characteristics of milk for the detection of the causative agents of bovine mastitis.

2. Develop and assess the potential supervised and unsupervised neural network models for the detection of major and minor pathogens that cause bovine mastitis.

3. Evaluate the impact of different proportions of infected to non-infected cases on the correct classification rate of the supervised neural network models as there are proportionately less infected cases in a herd compared to non-infected cases.

1.2 Research Justification

Traditionally, bacteriology is considered as the ‘gold standard’ for the identification of mastitis pathogens; however, this procedure requires a culture of suspected milk in the lab, making routine bacteriological monitoring of glands

expensive and time consuming. Another option is the use of milk parameter information obtained in-line to predict the causal agents of IMI, where diagnosis would be made while the animal is being milked. Mathematical algorithms used to detect changes in individual cows must be robust and able to deal with complex interactions, such as days in milk (DIM), season, cow age and breed (Hamann & Zecconi., 1998), making artificial neural networks (ANN) an ideal tool for analysing these type of multivariate data.

Advances in milking technology offer the possibility of measuring several milk parameters during the milking process. The proposed models offer the opportunity to use these milk parameters to detect the presence of particular types of mastitis pathogens, providing valuable information that can be used to manage mastitis in an efficient and effective manner.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Chapter Overview

This chapter reviews the literature related to mastitis. Sections 2.2 and 2.3 provide an overview of factors influencing susceptibility to bovine mastitis and the bovine immune system. Section 2.5 provides background to the mastitis diagnostic tests. Section 2.7 provides an introduction to ANNs. A brief overview of previous studies using ANN as a diagnostic tool for mastitis is given in section 2.9.

2.2 Factors Influencing Susceptibility to Bovine Mastitis

This section provides an overview of the various factors influencing the susceptibility of cows to mastitis. The dairy industry relies on increased milk production. Advances in technology (for milk removal from the udder) and genetic selection have led to an increased milk production per cow at the expense of increased susceptibility to mastitis (Sordillo, 2005). Susceptibility to mastitis increases with a decrease in the immunity of the host. Immune response of the cow is a manifestation of the genetic, environmental and physiological factors. Genetic selection that enhances milk production can impose metabolic stress and thus increase susceptibility to mastitis (Heringstad, Klemetsdal, & Steine, 2003). Poor farm management system and resulting farm environment can also increase mastitis susceptibility (Hogan & Smith, 2003). It is well known that increased milk production induces physiological stress that impairs the bovine immunity, and therefore, increases susceptibility to mastitis.

2.2.1 Genetic Factors

A number of genes regulate the bovine immune response. Information about the exact genes and phenotypes responsible for the immune response is very limited. For example, some authors agree that breeding cows with low SCC does not guarantee protection against mastitis (M.E Kehrli & Shuster, 1994; Schukken et

al., 1995; Suriyasathaporn, Schukken, Nielen, & Brand, 2000), although, other authors (Koivula, Mantysaari, Negussie, & Serenius, 2005) observed that susceptibility to mastitis was higher for cows with genetically higher SCC and that there was a linear relationship between SCC and CM.

Some studies focused on improving the physical characteristics (teat length and shape, teat canal width, udder depth) for increasing resistance to mastitis (Shook, 1989). According to a number of studies, it is possible to breed cows resistant to mastitis (Abdel-Azim et al., 2005; Uribe, Kennedy, Martin, & Kelton, 1995). Therefore, it can be concluded that heredity plays an important role in influencing the bovine immune response to pathogens.

2.2.2 Stage of Lactation

Many studies have reported higher incidence of mastitis in the early days of lactation, because at this stage the cow undergoes metabolic and physical stress (Abdel-Azim et al., 2005; Kelm et al., 1997). The majority of clinical mastitis (CM) cases occur in the early stage of lactation, although they can occur at any stage of lactation (Lund, Jensen, & Petersen, 1999).

2.2.3 Seasonal Effects

The incidence of mastitis is also influenced by seasonal variations (Abdel-Azim et al., 2005). This may be due to the variation of the causative organism due to season in the farm and its environment (Osteras, Solverod, & Reksen, 2006). Therefore, any model for mastitis diagnosis may benefit from incorporating the seasonal variations and their effects on disease prevalence.

2.2.4 Neuro-endocrine System

Higher incidence of mastitis is reported in the periparturent period that gives an indication of interaction between the neuro-endocrine and immune systems. For example, some hormones stimulate while others suppress the immune system. The somatotrophins have shown immunostimulatory effect (Burvenich et al.,

1999). This phenomenon may be explained by the action of the growth hormone on bone marrow in releasing the immune cells. Glucocorticosteroids, on the other hand, have immunosuppressant activity because they slow down the oxidative burst capacity of the Polymorphnuclear Neutrophils (PMN) thereby reducing their capacity to protect the mammary gland from the pathogens (Hoeben et al., 1999).

The length of day and night (photoperiod) can also influence the immune function. The prolactin level is influenced by the photoperiod that leads to photoperiodic effects on the immune system (Auchtung, Salak-Johnson, Morin, Mallard, & Dahl, 2004). Estrogens and estrogen-active compounds with forage may also be associated with increased susceptibility to mastitis (Zdunczyk , Zerbe, & Hoedemaker, 2003).

2.2.5 Lactation Number

With advancing age, the immune response to pathogens is reduced. In the old cows (in their 3rd to 5th Lactation) the immune response is weaker as compared to younger cows (1st or 2nd lactation) (Abdel-Azim et al., 2005; Harmon, 1994; Koivula et al., 2005; Sordillo, 2005).

2.2.6 Stress

Increased oxygen demand during early lactation and mammary growth leads to increase in the reactive oxygen species (ROS) (Sordillo, 2005). These reactive oxygen species are responsible for inducing tissue injury and creates oxidant stress.

2.2.7 Bacterial Resistance

Some major pathogenic bacteria like *Streptococcus uberis*, *Escherichia coli* and *Streptococcus dysgalactiae* have shown persistent adherence to the mammary gland (Bradley, 2002; Leigh, 1999). Possible explanation for this phenomenon

could be that these pathogens have developed a capability (or resistance) to evade the host's immune system.

2.2.8 Nutrition

Nutrition rich in elements that may enhance the immune system can greatly help the cow in combating the pathogenic attacks, but more research is needed in this area (Goff, 2006; Goff & Horst, 1997). In the early stages of lactation there is deficiency of protein and energy that may affect the cellular defense system, increasing the chances of mastitis (Barnouin & Chassagne, 1998). Proper nutrition at this period may improve the ability of the cow in fighting the pathogens.

2.3 Bovine Immune System

Nature has endowed every living organism with a certain potential to protect itself from the detrimental effects of the environment and other organisms. Cows also have developed special anatomical, cellular and soluble defense systems against the invading pathogens (Sordillo, 2005). The following figure illustrates the basic anatomical structure of the mammary gland:

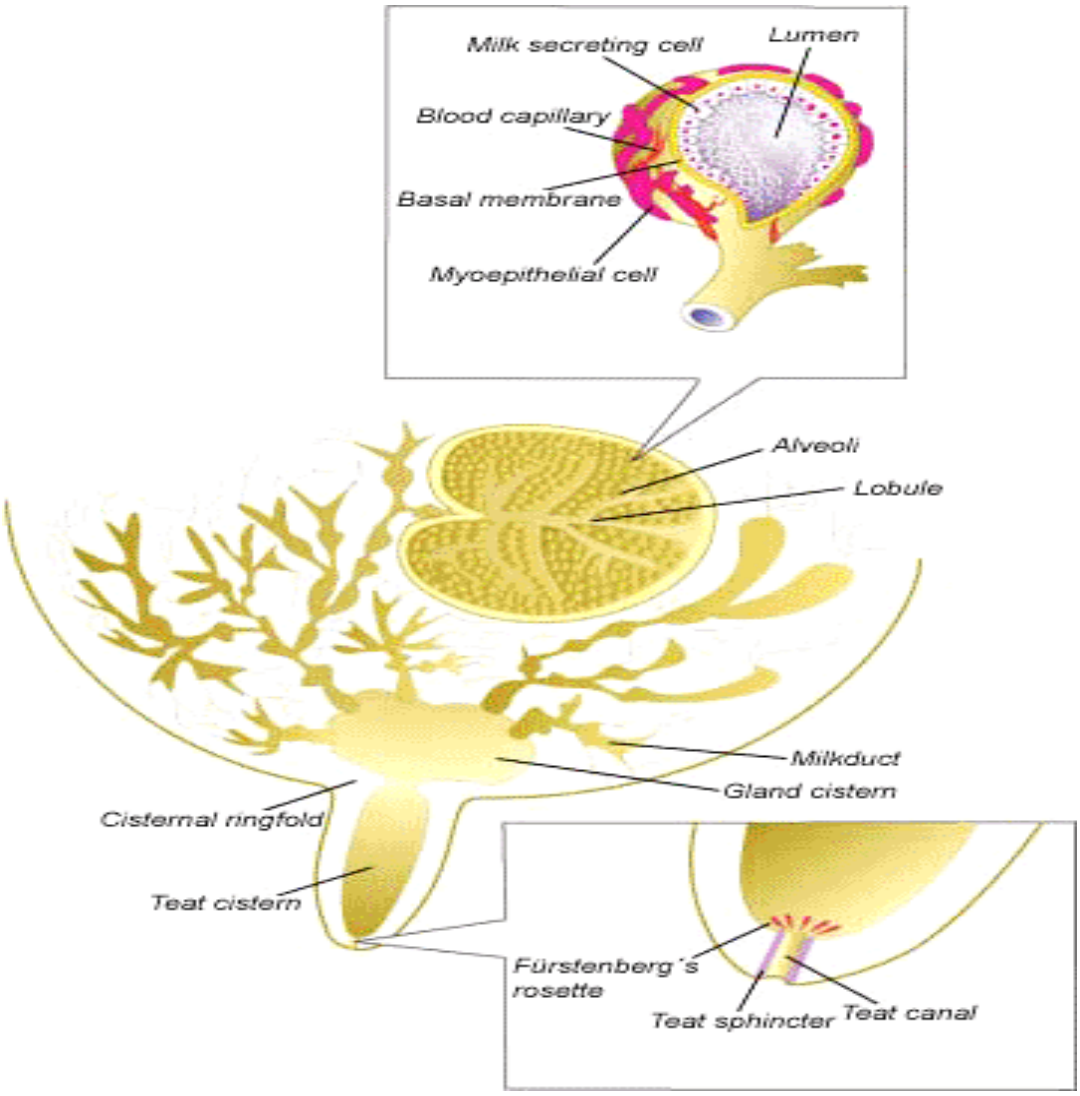

Figure 2.3.1 Anatomy of the Mammary Gland. Source (Delaval, 2006).

2.3.1 Anatomical Defense System

The first point of entry for bacteria is the teat opening (Figure 2.3.1). A tight closure of the teat opening by the sphincter muscle and accumulation of keratin ensures a physical barrier against the invading pathogens (Nickerson, 1987; Zecconi et al., 2000). Larger teat diameter is directly correlated to higher somatic cell count (Chrystal, Seykora, & Hansen, 1999). Particularly during the lactation period, there is increased milk production and associated dilatation of the teat mouth and teat canal that makes the mammary gland susceptible to mastitis (Nickerson, 1987; Oliver & Sordillo, 1988).

2.3.2 Cellular Defense System

When the pathogen enters the gland via the teat canal, then the cellular defense system - leukocytes (neutrophils, macrophages and lymphocytes) - of the host comes into play. Bacterial infection causes the release of inflammatory mediators from the gland that causes the leukocytes to move from the blood to the gland causing phagocytosis of bacteria (Persson, Larsson, & Sandgren, 1993). The severity and duration of infection depends on the availability and activity of these leukocytes at the site of infection. According to some scientists the initial response to an infection is the increased production of neutrophils and comprises 90% of the total cellular defense system (Paape, Bannerman, Zhao, & Lee, 2003). This type of cellular defense system is also known as non-specific or innate immunity (Sordillo, 2005). However, during the periparturent period, many functions of the neutrophils are altered or disabled that causes reduced immunity against bacteria making the cow highly susceptible to bacterial infections (Burton & Erskine, 2003; M. E Kehrli, Nonnecke, & Roth, 1989).

Macrophages also exhibit innate as well as acquired immune response to bacterial infection. Lymphocytes are of two types: T-Lymphocytes and B-Lymphocytes; and they have the capability to remember and identify a particular pathogen and either destroy the pathogen themselves or trigger other immune cells to attack the pathogens.

2.3.3 Soluble Defense Systems

Like the cellular defense system, these are also of two types: innate and acquired. The primary function of these molecules is to increase the phagocytosis potential of neutrophils and macrophages. These are immunoglobulins (Ig) of different types (IgG1, IgG2, IgM) acting as opsonins[1]. During the early stages of lactation their activity and levels are altered which might explain the increase in incidence of mastitis during the periparturent period (Sordillo, 2005).

[1] These are molecules that increase the binding potential of the phagocyte with the pathogen.

2.3.4 Summary

This section provided a brief introduction to factors affecting susceptibility of dairy cattle to mastitis. In addition, the bovine immune system was briefly explored. Like any other biological phenomenon, it is a highly complex system. Next section provides an overview of the various diagnostic methodologies for the detection of mastitis.

2.4 Diagnosis of Bovine Mastitis

Mastitis causes a number of changes in milk parameters (i.e. biochemical milk profile). By measuring the chemical (i.e. citrate, phosphate, potassium, sodium and beta-hydroxybutyric acid) and compositional (i.e. lactose, somatic cells, minerals and enzymes) attributes of milk, it is possible to provide information about the health status of a cow (Hamann & Kromker, 1997).

Physical signs (swelling of the udder, clots and color changes of milk) of clinical mastitis (CM) can be observed with the naked eye. But it is extremely difficult to observe the signs of sub-clinical mastitis (SCM). Bacteriological examination can provide an accurate detection of SCM. Some of the milk parameters like somatic cell count (SCC), electrical conductivity (EC), milk amyloid A (MMA) (an acute phase protein which acts as a signal to the immune system) and lactate (a bacterial metabolite) have been used in a number of studies for the detection of SCM. Some milk parameters show significant deviation from the normal levels, and therefore, can be used for the detection of the inflammatory response of the udder to mastitis (Hamann & Kromker, 1997).

2.4.1 Bacterial Culture (BC)

The main cause of mastitis is bacterial infection and therefore their accurate detection is the most effective method for the diagnosis of mastitis. Bacteriological examination of milk samples provide valid diagnosis for correct treatment (Obritzhauser, Deutz, & Fuchs, 1995). Detection of the type of pathogen can be used to design appropriate treatment and management strategies

at the cow and farm level. However, this is a very expensive and time consuming procedure.

2.4.2 Somatic Cell Count (SCC)

The level of SCC in milk reflects the response of the udder to an infection. High correlation exists between CM and higher levels of SCC (Koivula et al., 2005; Mrode, Swanson, & Winters, 1998; Schepers, Lam, Schukken, Wilmink, & Hanekamp, 1997; Suriyasathaporn et al., 2000). The pattern of SCC during the mastitis period may provide information about the type of pathogen involved (Heald, Kim, Sischo, Cooper, & Wolfgang, 2000). However, SCC is not only influenced by mastitis but also by cow's genetics (Section 2.2.1), stage of lactation (Section 2.2.2) and age of the cow (Section 2.2.5).

Tests like California Mastitis Test (CMT) and Wisconsin Mastitis Test (WMT) involve the addition of a reagent to the milk sample and the resultant viscosity provides a rough estimate of the SCC (Higher the SCC, higher the viscosity and vice versa) (Whyte, Walmsley, Liew, Claycomb, & Mein, 2005). These are simple and economical cow side tests for the diagnosis of mastitis.

2.4.3 Electrical Conductivity (EC) or Electrical Resistance (ER)

Electrical conductivity or electrical resistance measures the ionic changes resulting from damage caused by bacteria to the alveoli (Kitchen, 1981). The EC or ER comes into play at a later stage of infection when the blood-milk barrier is broken. Like SCC, EC or ER of milk may also reflect the changes associated with a bacterial infection (Nielen, Deluyker, Schukken, & Brand, 1992). In one study, the sensitivity of EC in detection of SCM was 95% (Lein & Wan, 2000). In another study, the specificity (correct identification of healthy cases) and sensitivity (correct identification of infected cases) of a handheld EC meter was 19% and 91%, respectively (Mansell & Seguya, 2003). Like SCC, it is also a simple and economical cow side test. However, the EC of milk varies from cow to cow within a herd, from herd to herd and from breed to breed. EC also varies

over the course of lactation. EC or ER even varies during milking (i.e. foremilk, mid flow and stripping) (Bansal, Hamann, Grabowski, & Singh, 2005). Nutrition type, fat content and temperature of milk can also affect the EC measurements. Therefore, deviation of EC from a normal quarter of a particular cow may be more informative than comparing the absolute EC values (Wang & Samarasinghe, 2005; Woolford, Williamson, & Henderson, 1998).

2.4.4 Milk Lactate

When bacteria enter the teat canal and start to increase in population, bacterial metabolites are released. Lactate is one of those metabolites and its detection in milk can be used as a diagnostic tool for CM and SCM (Davis et al., 2004). In their study, lactate concentration was positively correlated with the SCC. However, in one case, the SCC was high in one clinically ill cow but the lactate concentration was very low indicating the weakness of lactate as a diagnostic tool. The above study was conducted on a small number of animals and the authors concluded that more large scale studies are needed to establish the usefulness of lactate concentration in milk as a mastitis diagnostic tool (Davis et al., 2004).

2.4.5 Milk Amyloid A (MAA) or Acute Phase Proteins (APP)

The introduction of bacteria to the udder activates the immune response of the cow. During this period MAA/APP is released which can be detected in the milk (Eckersall et al., 2006). Early detection of the presence of MAA or APP can be used as a diagnostic tool before an increase in the SCC occurs. According to one study, a positive correlation between MAA or APP and SCC was found (Sensortech, 2006).

2.4.6 Milk Protein and Fat Content

Mastitis also affects protein (Urech, Puhan, & Schallibaum, 1999) and fat (King, 1978) composition of milk. Measuring these parameters can also provide information about the health status of a cow.

2.4.7 Summary

In this section, we explored the various methods available for the diagnosis of mastitis. Their strengths and weaknesses were also discussed. Bacteriological examination of suspected milk is the most effective way to detect the type of pathogen to devise relevant treatment and management strategies. However, it is a costly and time consuming procedure. New in-line measurement technologies offer the opportunity to measure many milk parameters directly during milking. These parameters are affected when the mammary gland is invaded by pathogens. These changes can be used to detect the type of infection. The next section provides a brief introduction to ANNs.

2.5 Artificial Neural Networks

2.5.1 Introduction

This section provides a brief introduction to artificial neural networks (ANN). Those interested in details are advised to read "Neural Networks for Applied Sciences and Engineering" by Samarasinghe, (2006).

Biological, chemical and physical processes on the surface of the planet are highly complex and interdependent. The ANNs are capable of handling complex and non-linear relationships (Hair, Anderson, Tatham, & Black, 1995; Samarasinghe, 2006). For example, crop production and quality depends on biological (cell division, genetics, reproduction, growth, death), biochemical (photosynthesis; proteins, carbohydrates, and lipids synthesis) and physical (respiration, heat transfer, solar radiation, air and soil temperatures, transpiration, evapotranspiration) factors. To understand and improve crop production and quality, scientists need a systematic approach that can integrate and model these linear and nonlinear phenomena with high reliability. The ANNs are capable of reliably integrating and modeling the behavior of such complex interactions and therefore, can solve a number of problems that are difficult to solve with conventional modeling approaches.

The ANNs (hereafter called neural networks) are interconnected networks of artificial neurons that acquire knowledge by processing information in a manner analogous to the human brain. The basic unit of a neural network is an artificial neuron. The functionality of a single neuron is explored in Figure 2.5.1.1.

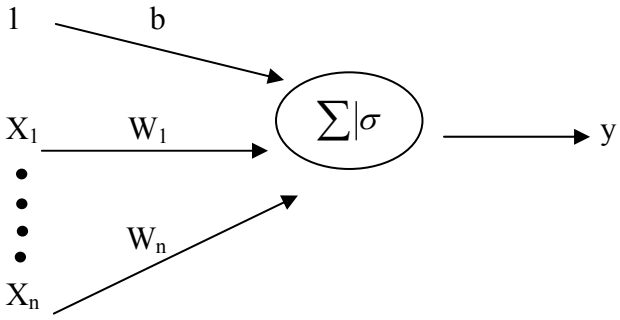

Figure 2.5.1.1 Structure and functionality of a single neuron.

The inputs to a neuron (X_1……X_n) are multiplied by a set of weights (W_1…..W_n). Input of 1 represents a bias and accounts for the factors not considered by the set of inputs. These weighted inputs are summed together and set into a nonlinear function σ. The commonly used functions are logistic, hyperbolic-tangent, arc tangent, Gaussian and sine. When a number of these neurons are set together in a network, it forms an ANN as shown in the Figures 2.5.2.1 and 2.5.3.1 below. Neural networks may be supervised or unsupervised depending on the learning paradigm being used. They are explored in the following sections.

2.5.2 Supervised Neural Networks

In the case of supervised learning, the output for a given set of inputs is used in the training process (Rumelhart, Bernard, & Michael, 1994). A typical supervised neural network is shown in Figure 2.5.2.1; the inputs are multiplied by a set of weights, and passed through the hidden layer using one of the activation functions discussed earlier. There may be one or many hidden layers and neurons in the network, depending on the complexity of a problem. However, a large number of hidden layers and/or neurons is not necessarily a criterion for the successful solution to a problem. The optimum number of hidden layers and neurons has to be found through a search process. When the inputs are passed through the hidden layer or layers, the output of the network (y_1….y_n) is produced by the output layer.

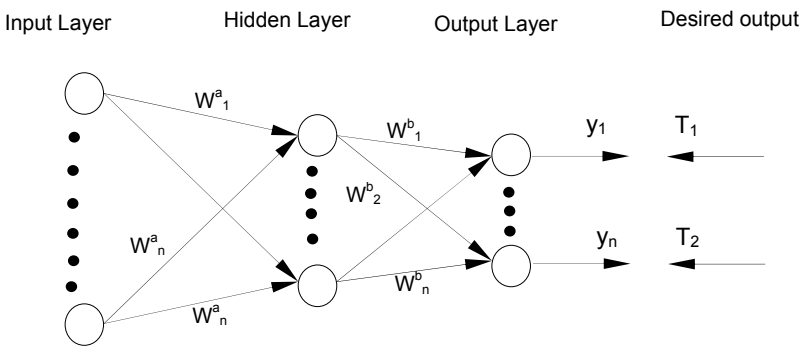

Figure 2.5.2.1 Topology of a supervised neural network. Adopted from Samarasinghe, (2006).

This output is compared with the desired output (T_1…..T_n); here, a cost function is applied that calculates the difference between the network output and the desired output. This error of the network is then back-propagated, that leads to changes in the connection weights of the network through delta rule, a powerful gradient based weight adaptation method. This is basically an iterative process and is repeated for a number of iterations. When the error is reduced to an acceptable level, then the training is stopped. This trained network can be used to identify similar patterns in a new situation and obtain the appropriate prediction or classification.

In supervised neural network modeling, a dataset is typically divided into three sets: training, calibration or test and validation. A network is trained with the training set while test or calibration set is used intermittently throughout the training to assess the generalization ability of the model. Training is stopped at the point where the generalization error is the minimum. The trained network is further assessed by the validation set that the network has not seen before. The statistical indicators of performance (R^2, Mean Square Error etc) of the trained network on the validation set is typically presented as measure of accuracy of the model.

2.5.3 Unsupervised Neural Networks

These type of neural networks learn without being shown any target output. Self Organising Feature Map (SOFM) (Kohonen, 1998) is one example of unsupervised neural networks. This type of network is composed of two layers (Figure 2.5.3.1), the input layer representing the input variables (X_1…….X_n) and an output layer of neurons which are trained during the training process. Training involves the adaptation of the connection weights between the input and the output layer. After adaptation or training the output neuron become self organized and a feature map is formed between the inputs and output neurons.

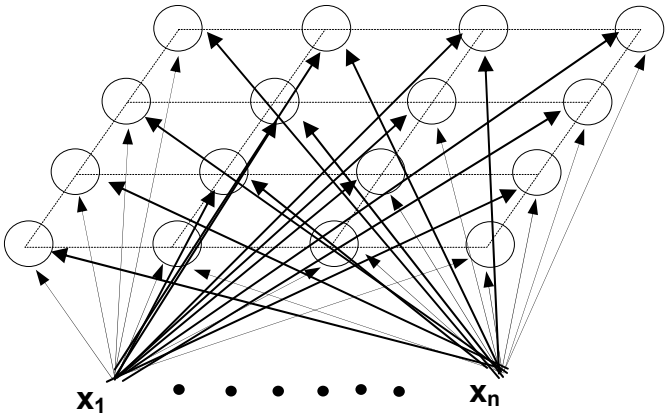

Figure 2.5.3.1 Topology of an unsupervised neural network. Adopted from Samarasinghe, (2006).

In the SOFM network, small random values are used to initialize the connection weights. These weights are adopted during the training process. First the winning neuron is identified by calculating the distance of each neuron to the input vector. The most commonly used method for distance calculation is the Euclidean distance. The neuron that is closer to the input vector is the winner and will have higher activation or weight change as compared to one that is farther from it. In the SOFM network, the weight of the winner and its neighboring neurons are updated, so that they move closer to the input vector using a learning rate and neighbor strength (Samarasinghe, 2006).

Neighboring neurons that are closer to the winner get higher weight changes as compared to distant neighbors using a neighbor strength (NS) function. The most commonly used NS functions are Gaussian, Linear and Exponential. The NS is kept large at the beginning of training to ensure proper organisation of the weight vectors. It is essential to reduce the NS with advancing iterations; this will further improve the organisation of the weight vectors. The NS is reduced using linear or exponential decay functions.

During training, the learning rate is kept high at the start and is reduced with iterations; Linear and Exponential learning rate decay functions are commonly used for this purpose. The trained neurons (i.e. their weights) represent the input data in a compressed form. In other words, the multidimensional data is represented in a two dimensional form, which can be easily analysed and evaluated.

In the SOM model development, a data set is divided into two sets: training and validation. The training set is used to train the network by adjusting weights and the validation set is used to assess the generalization ability of the model when assigned to unseen data. Once the trained map is clustered, then we can classify new data based on its cluster membership on the map.

2.5.4 Summary

This section provided a brief introduction to ANNs. The two main neural network learning paradigms were briefly discussed. They learn from past experience and are able to model complex phenomena. Neural networks can be applied for function approximation, classification, pattern recognition, time series modelling and, data processing and clustering. They are capable of modeling linear and non-linear phenomenon with high precision. They have been used in situations such as, automatic aircraft control, credit card fraud detection, medical diagnosis, face recognition and long term river flow forecasting.

The next section provides a review of the literature related to the use of neural networks for the diagnosis of mastitis.

2.6 The Use of the Artificial Neural Networks (ANNs) for Mastitis Diagnosis

ANNs have been used in a limited number of studies for the diagnosis of mastitis. This section provides an overview of the relevant previous studies with respect to the number and type of variables used and analysis of results. The relative strengths and weaknesses of various models with respect to their sensitivity and specificity are also discussed.

In one study (Nielen, Schukken, Brand, Haring, & Ferwerdavanzonneveld, 1995), supervised neural networks were used to analyse in-line milking parlor data to detect CM in dairy cattle. They used automatically collected EC data as input to the ANN to classify mastitic and healthy quarters. Sensitivity (correct identification of diseased cases) and specificity (correct identification of healthy cases) of the model was approximately 75% and 90 %, respectively. The network was trained with 13 mastitic and 17 healthy quarters. The model correctly classified 21 of 38 mastitic quarters (On Validation Set), and 34 of 38 healthy quarters. They concluded that the ANN was able to classify healthy and mastitic quarters without any normalization for cow or herd level. However, according to other studies (Wang & Samarasinghe, 2005; Woolford et al., 1998) it was recognition of cow to cow variation in the absolute EC values that provided better discrimination between mastitic and healthy quarters. Nielen et. al. (1995) also suggested that in future studies large data sets and other mastitis related variables should be included in building more robust models.

Yang et. al. (1999) used a supervised neural network to assess its ability in differentiating between healthy and diseased cows. They used SCC, lactation number, milk yield, days in milk, herd size on test day, mean SCC for herd, season of calving and milk components. They achieved an overall accuracy of

86% in classifying mastitic and healthy cows. They also found that different ratios of mastitic to healthy records (1:1, 1:10 and 1:300) and different output threshold values (0-1, 0.5 in most cases) had the same effect on the learning ability of the ANNs. In their research, the EC of milk was not used. They concluded that better data pre-processing and more accurate inputs for the ANN could have led to more accurate results.

Heald et. al. (2000) used Dairy Herd Improvement Association (DHIA) and field survey data to categorize bacterial causes of mastitis in dairy herds using supervised ANN and linear discriminant analysis (LDA). The data used as inputs for the models consisted of individual cow data from the DHIA test day (Average SCC during current lactation, SCC of test day, milk production, lactation number, days in milk, number of severe test days i.e. SCC > $4.9*10^6$, and SCC as % of bulk tank SCC) and a survey data related to herd management practices (Bulk tank standard plate count, cases of CM, cow housing and bedding, milking time practice of the herd, status of pre-dipping and average milking time). The four outputs were the foremilk bacteriological results for each quarter (Environmental, Contagious, Others and non-infected). The overall correct classification results were in the range 57 to 71% for ANNs as compared to 42 to 57% for Linear Discriminant Analysis (LDA). Sensitivity was 52% and specificity was 83% for the ANN with 100 cows, 45% and 80%, respectively, for the model with 200 cows, and 39% and 79%, respectively, for the model with 300 cows.

The results of Heald et. al. (2000) study indicates that overall, ANN performed better compared to LDA. The specificity of all the neural network models was high, but their sensitivity was low. One reason may be the type of data and variables used. The DHIA data were collected on a monthly basis and the bacteriological data was collected only once which might not have provided sufficient information for the ANN to identify patterns in the training data set and thus performed poorly on the validation data set. The EC or ER (which may have

improved the model results) were not used in their research. Due to the overall poor classification accuracy, the applicability of the model at the farm level may be low.

Wang and Samarasinghe, (2006) compared the efficacy of supervised ANN and unsupervised ANN and LDA for the classification of CM and healthy cows. They used two versions of cow and herd normalised EC (*EcMax*-peak electrical conductivity and *EcDV*-maximum relative deviation of EC among four quarters for a cow) and cow and herd normalized quarter yield fraction (*QYF*: Ratio of milk production of a quarter to the sum total milk production from all four quarters). The correlation analysis revealed high correlation among the input variables; therefore, they used principle component analysis (PCA) and used principle components instead of variables in their original form. The classification results using LDA showed a sensitivity of 81% and specificity of 100%. For the Multi Layer Perceptron (MLP) network (a type of supervised ANN), sensitivity was 84% and specificity was 100%. Therefore, there was no significant difference between the two modeling approaches. However, for the self organizing feature map (SOFM) model (a type of unsupervised ANN) the sensitivity was 95% and specificity was 100% indicating high efficacy of SOFM compared to MLP and LDA (Wang & Samarasinghe, 2005).

Nielen et al. (1995) explored the prediction accuracy of logistic regression model (LRM) and multiplayer perceptron neural networks for SCM using in-line milking data. The variables used in their research were, EC per quarter (measured every 5 seconds), Lactation Number (LN), days in milk (DIM), and milk production per cow. They pre-processed the input variables before presenting to the models. Milk production data were used to calculate the expected production in each milking. Parity and DIM were divided into groups. The EC level for a cow was calculated from the individual quarter's measurements. The first dataset contained 5139 classified as healthy cow milkings and 1244 classified as SCM. The second dataset had 4614 cow milkings classified as healthy and 1080 classified as SCM. The SCC measurements (measured twice weekly) were used

to define healthy periods (four consecutive cow readings of SCC levels <200 × 10^3 cells/ml). Sub-clinical periods were defined for SCC levels >500 × 10^3 cells/ml, for a minimum of 1 week (14 milkings) (Nielen, Schukken, Brand, Deluyker, & Maatje, 1995). Two datasets were created for analysis.

If the signals were higher than the SCM threshold for more that 6 signals out of 14, then the period was termed SCM and otherwise termed healthy. Sensitivity for SCM periods with the specified threshold was 54% for the logistic regression model and 66% for neural network model. Specificity for healthy periods was 92% and 80%, respectively, for the two models (Nielen, Schukken, Brand, Deluyker et al., 1995). Their research indicated that historical EC data can be used to assess the health status of individual cows.

López-Benavides (2004) developed a Self Organising Feature Map (SOFM) model to cluster quarter milk samples into different health categories for the diagnosis of mastitis. The variables used in the study were EC, SCC, protein percentage (PP) fat percentage (FP), and bacteriological growth results for each of every quarter milk samples collected on a weekly basis. The data pre-processing led to a number of new variables. EC and Inter-Quarter Ratio[2] (IQR) for each quarter were used to calculate a conductivity index[3] (CI) and Somatic Cell Score[4] (SCS) was derived from SCC. The composite milk index[5] (CMI) was derived from the original variables and their derivatives. The SOFM model used

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[2] Inter Quarter Ratio $IQR_i = \frac{\sum_{j=1}^{4} EC_j}{EC_i}$ Where *i*=Quarter number ; *j*=iteration number of quarter

[3] Conductivity Index $CI_i = 2 + \left[\left\{ \frac{EC_i}{100} \right\} - IQR_i \right]$

[4] Somatic Cell Score $SCS_i = \left[\log_2 \frac{SCC_i}{100} \right] + 3$ (SCC of herd test record used in units of 10^3)

[5] Composite Milk Index $CMI_i = IQR_i + FP_i + PP_i + SCS_i + CI_i + A_i + B_i + C_i + D_i + E_i$
Where FP=Fat%; PP=Protein %; A_i= ln (PP_i * FP_i); B_i=(CI_i * SCS_i); C_i=(CI_i * FP_i); D_i=(CI_i * PP_i); E_i=(A_i*B_i)

two inputs, CMI and CI for the classification of quarters into arbitrarily defined health categories (healthy, moderately ill, ill and severely ill).

In the study, it was found that as the health status changed from healthy to severely ill, somatic cell count, conductivity index, and composite milk index changed significantly ($p<0.001$). The percentage of non-infected cases in the healthy category was 93%, moderately ill 92%, ill 90% and severely ill 50%. About 8% of cases in the healthy category were infected due to small or no changes in their milk parameters.

2.6.1 Summary

This section explored the literature related to the use of ANNs (and statistical methods where relevant) in mastitis detection. In only one study (Heald et. al. 2000), neural networks were used to classify the bacterial causes of mastitis with limited success. In their study, electrical conductivity or electrical resistance, protein and fat percentages were not studied. These are important milk parameters and may provide useful information for detecting the type of infection.

The proposed study is an effort in developing more robust models for the detection of major and minor infections that cause mastitis. A number of milk parameters along with other important variables such as days in milk, lactation number and sire number will be used in this research.

The next chapter explores the materials and methods employed to achieve the proposed objectives.

CHAPTER 3

3. MATERIALS AND METHODS

3.1 Introduction

This chapter explores the materials and methods used to achieve the research objectives. Sections 3.2 and 3.3 provide information about the experimental data and the data preprocessing steps followed to prepare the data for advanced analysis. Sections 3.4 and 3.5 provide details of the data partitioning procedures. Section 3.6 describes the analysis performed on the preprocessed data and section 3.7 explores the configurations of the neural network models.

3.2 Experimental Data

Data analysed in this research were collected at the Lincoln University dairy farm, in Canterbury, New Zealand, from August to November 2002. Starting at calving, quarter foremilk samples were collected weekly from 112 cows for a period of 14 weeks, resulting in 4852 samples with complete records for SCC, ER, FP, PP and bacteriological status (Lopez-Benavides, 2004). Records with missing values for any one of the milk parameters were not used in the analysis. Similarly, records with unusually very high or very low values for a particular infection status were considered as outliers and were not used in the analysis. Cows were daughters of three different sires. In summary, SCC, FP and PP were measured using CombiFoss 500 (Foss Electric, Denmark) at Livestock Improvement Corporation Hamilton, New Zealand; ER was measured using the Draminski Mastitis Detector (Draminski, Warsaw, Poland), and bacteriological analysis of milk samples was performed according to the standard guidelines (NMC, 1999) at the Dexcel Mastitis Research Lab in Hamilton, New Zealand.

3.3 Data pre-processing

The following transformations were performed on the raw data before advanced analysis; due to its skewed distribution SCC (1000/ml) was transformed into Somatic Cell Score (SCS), as shown in equation [1] (Dabdoub & Shook, 1984; Lopez-Benavides, 2004).

$$SCS_i = \left[\log_2 \frac{SCC_i}{100} \right] + 5 \qquad [1]$$

Electrical Resistance Index (ERI) for each quarter was developed to account for within and between cow variations as shown in equation [2]. From various attempts at normalization, the relationship shown in equation [2] was adopted as it provided better separation of infected and non-infected quarters than the approaches reported in literature and several others tested in this study. Individual quarter ERI was derived from cow ER values, which is the relationship of the individual quarter ER to the total ER for a cow, and to the maximum ER of a quarter on the test day.

$$ERI_i = \frac{ER_i}{ER_{\max}} - \frac{ER_i}{\sum_i^4 ER_i} \qquad [2]$$

Where ER_i is the ER of quarter i, ER_{max} is the maximum ER observed from an individual cow and $\sum_i^4 ER_i$ is the total ER from all four quarters on the test day.

To account for the effects of stage of lactation on milk parameters with respect to stage of lactation or days in milk (DIM), data were divided into three ranges (Range 1 = 1 to 30 DIM; Range 2 = 31 to 57 DIM and Range 3 = 58 to 125 DIM). Similarly, cow variation was accounted for by the sire family (SN) from which the cow originated and the lactation number (LN) of each cow. Bacteriological status (BS) was coded according to bacteriology of each case (no infection = NI; minor pathogen infection = MNI; major pathogen infection = MJI).

3.4 Data Partitioning for Unsupervised Neural Network (USNN) Model

In order to develop the USNN model for the detection of infection status of a quarter, the dataset was partitioned into training and validation sets. The training set was used to develop the USNN model and the validation set to assess its generalization capability. The training set was obtained by random extraction of an approximately equal amount of data from each BS using STATISTICA, (2006). This consisted of 50 NI, 43 MNI and 37 MJI cases. The remaining data containing 4411 NI, 301 MNI and 10 MJI cases were used for validating the USNN model.

3.5 Data Partitioning for Supervised Neural Network (SNN) Model

Data set for SNN models was partitioned into training, calibration and validation sets. The two main objectives for the development of the SNN models were, (1) to evaluate the potential of this modelling paradigm in detecting the infection status of a quarter, and (2) to evaluate the effects of different proportions of infected and non-infected cases in the training data set on the correct classification rate of the model.

Three data sets were extracted randomly using STATISTICA, (2006). The training and calibration sets were used to develop the model and the validation set was used to assess its accuracy on unseen or new data. The datasets were extracted as shown in Table 3.5.1.

Table 3.5.1 Datasets for training, calibration and validation of supervised neural networks (SNN).

Infected vs Non-Infected Ratio	Minor (n)			Major (n)			Non Infected (n)			Total (n)			Grand Total
	Training	Calibration	Validation	Training	Calibration	Validation	Training	Calibration	Validation	**Training**	**Calibration**	**Validation**	
1:1	217	56	71	33	4	10	260	65	82	510	125	163	**798**
1:2	217	56	71	33	4	10	496	113	170	746	173	251	**1170**
1:4	217	56	71	33	4	10	1009	230	313	1259	290	394	**1943**
1:10	217	56	71	33	4	10	2496	601	780	2746	661	861	**4268**

3.6 Data Analysis

3.6.1 Introduction

Before developing any model, it is important to identify any linear and non-linear relationships between the input and output variables. To identify relationships between variables, data were analysed using statistical (MINITAB, 2003 ; STATISTICA, 2006) and multivariate data visualization (XmdvTool, 2006) software. Correlation analysis between variables was conducted to identify the most influential input variables. The principle component analysis (PCA) was carried out to eliminate multicollinearity between the input variables. Configurations of the best neural network models are also described in this section.

3.6.2 Correlation Analysis

Correlations ($p < 0.05$) were found between the input and output variables in the training data set for the USNN model (Table 3.6.2.1). The SCS and ERI were strongly correlated to BS (r = 0.86, r = -0.59, respectively). Moderate correlation was observed between PP and BS (r = 0.33), while no correlation was observed

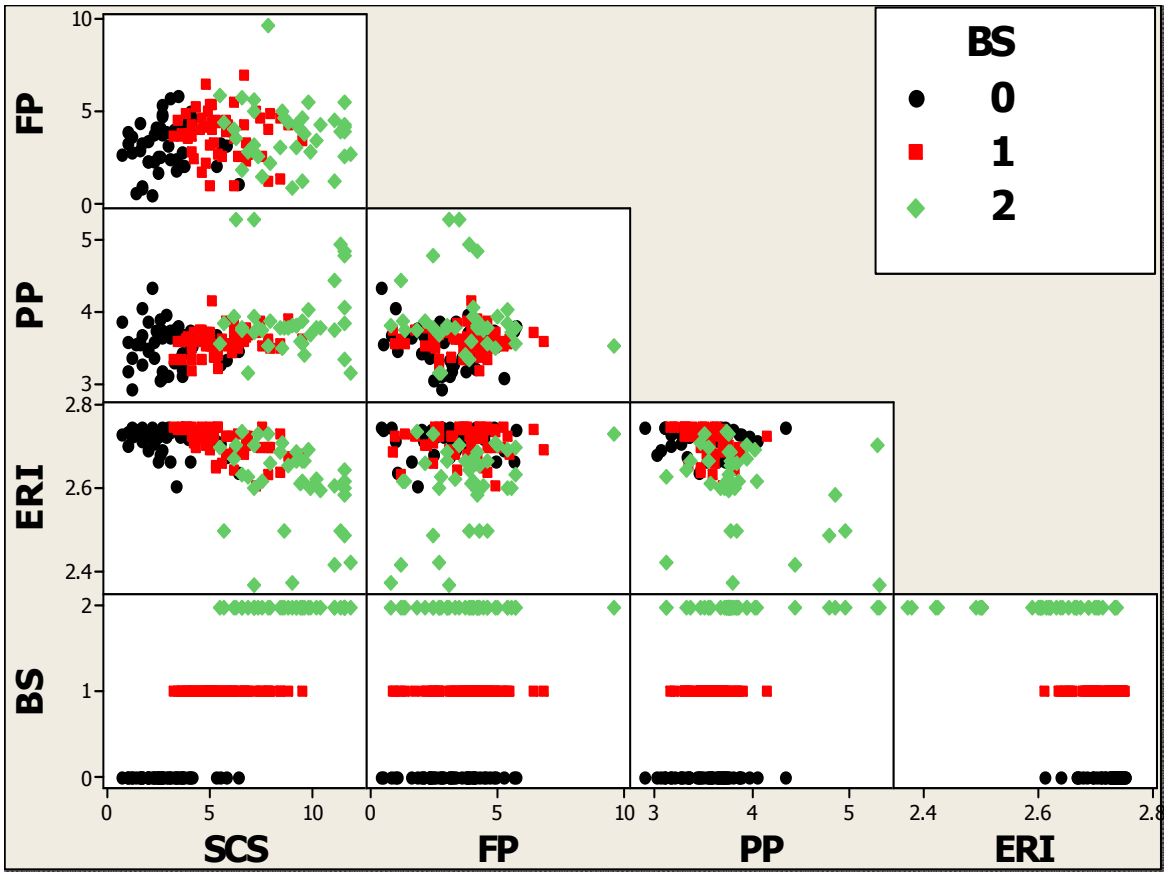
between FP and BS (r = 0.07). However, strong correlations between ERI and SCS (r = -0.65), and moderate correlations between PP and SCS (r = 0.32), and PP and ERI (r = -0.35) were observed. Weak correlations were observed between FP and ERI (r = 0.18), DIM and ERI (r = 0.19); and DIM and PP (r = -0.22). No significant correlations were observed for SN and LN.

Table: 3.6.2.1 Correlations between the Input and Output variables [Training dataset for unsupervised neural network model (n=130)].

Key BS = Bacteriological State; SCS = Somatic Cell Score; ERI = Electrical Resistance Index; PP = Protein Percentage; FP = Fat Percentage; * $p < 0.05$

	SCS	ERI	PP	FP	DIM	LN	BS
SCS							**0.86***
ERI	**-0.65***						**-0.59***
PP	**0.32***	**-0.35***					**0.33***
FP	0.12	**0.18***	0.09				0.07
DIM	-0.08	**0.19***	**-0.22***	0.07			-0.08
LN	-0.01	0.09	-0.12	0.12	0.08		-0.03
SN	-0.03	0.13	-0.03	-0.04	-0.01	-0.12	0.06

The following scatter plot (Figure 3.6.2.1) shows the relationships between the input and output variables. There are no strong non-linear relationships between the variables. SCS and ERI provide good discrimination between the three bacteriological states, while PP and FP are weak discriminators.



Key: BS = Bacteriological State [No Infection = 0, Minor Infection = 1 and Major Infection =2]; SCS = Somatic Cell Score; ERI = Electrical Resistance Index; PP = Protein Percentage; FP = Fat Percentage.

Figure 3.6.2.1 Scatter plots of SCS, ERI, FP, PP and BS. [Training data set for unsupervised neural network model (n=130)].

3.6.3 Analysis of Variance of Milk Parameters for the three Bacteriological States

To analyze differences in mean values of milk parameters for the three bacteriological states, One Way ANOVA was used (Table 3.6.3.1, Figures 3.6.3.1, 3.6.3.2, 3.6.3.3 & 3.6.3.4). Mean SCS increased (p = 0.001) linearly from non-infected to minor and major infected cases. A negative linear trend was observed for the electrical resistance index (ERI), but only MJI cases were different. The protein percentage (PP) was higher only for the major infected cases. The means for fat percentage (FP) were not different between the three bacteriological states.

Table 3.6.3.1 Milk parameters means (±SEM) for each bacteriological status for the training dataset (n=130).

	Bacteriological Status			
Milk Parameters	**NI**	**MNI**	**MJI**	**(p<0.001)**
Somatic cell score	2.71± 0.21	6.05 ± 0.23	9.04 ± 0.24	All states
Electrical resistance index	0.72 ± 0.01	0.69 ± 0.01	0.60 ± 0.01	MJI only
Milk protein (%)	3.54 ± 0.05	3.60 ± 0.05	3.86 ± 0.06	MJI only
Milk fat (%)	3.53 ± 0.22	4.04 ± 0.24	3.76 ± 0.26	None

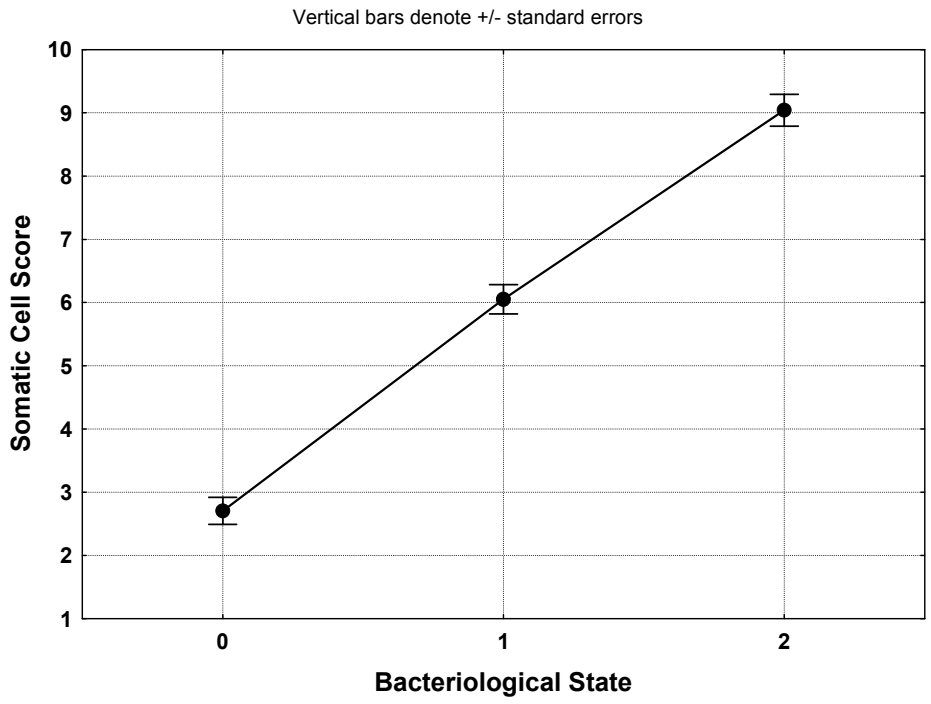


Figure 3.6.3.1 Least square means difference of somatic cell score between the three bacteriological states for training data set (n=130).

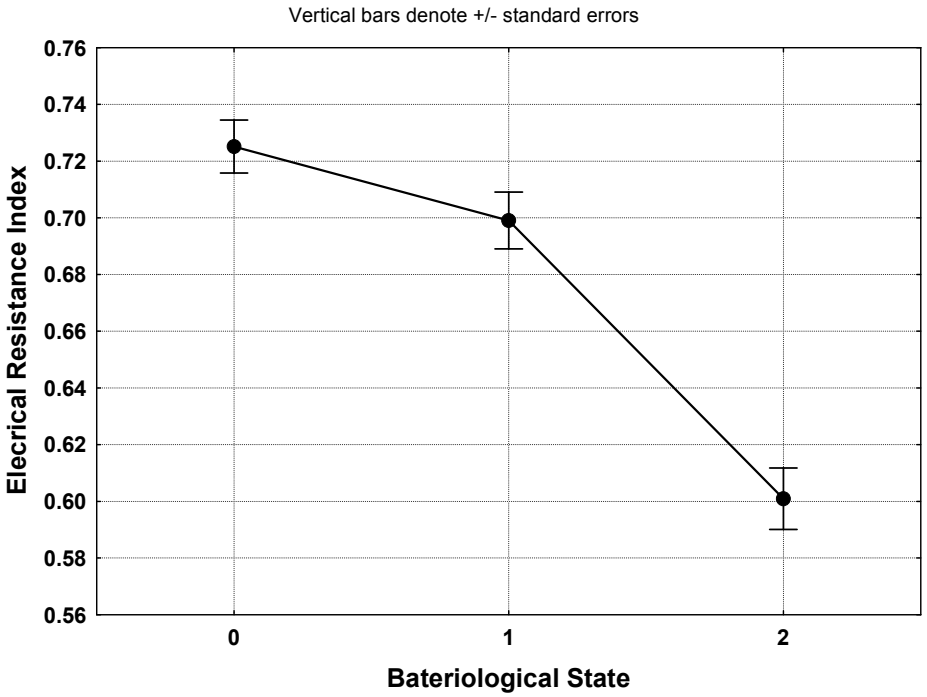


Figure 3.6.3.2 **Least square means difference of electrical resistance index between the three bacteriological states for training dataset (n=130).**

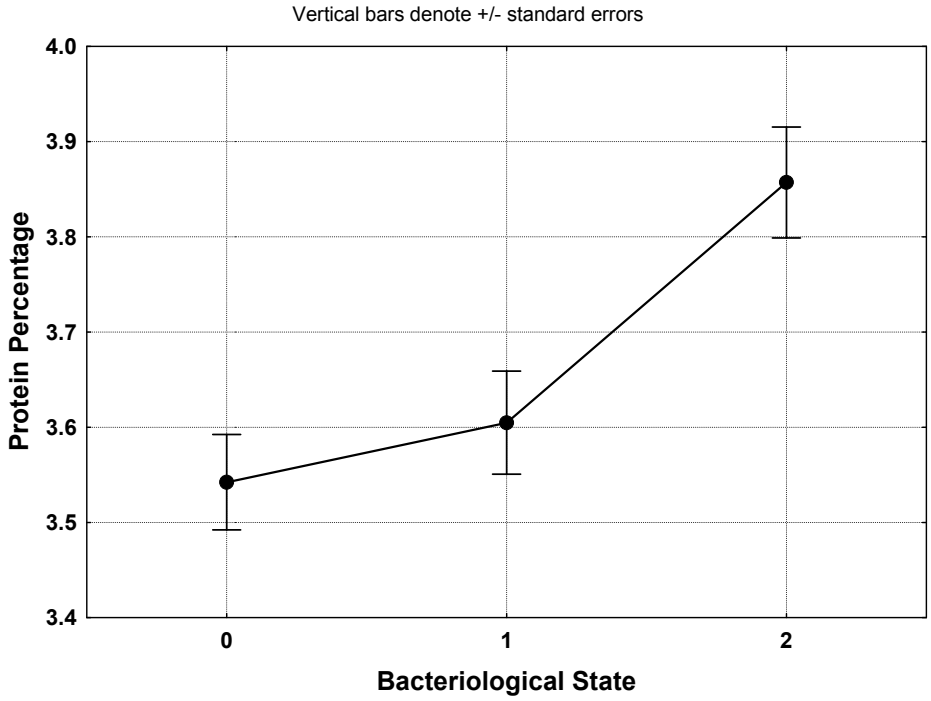


Figure 3.6.3.3 **Least square means difference of protein percentage between the three bacteriological states for training dataset (n=130).**

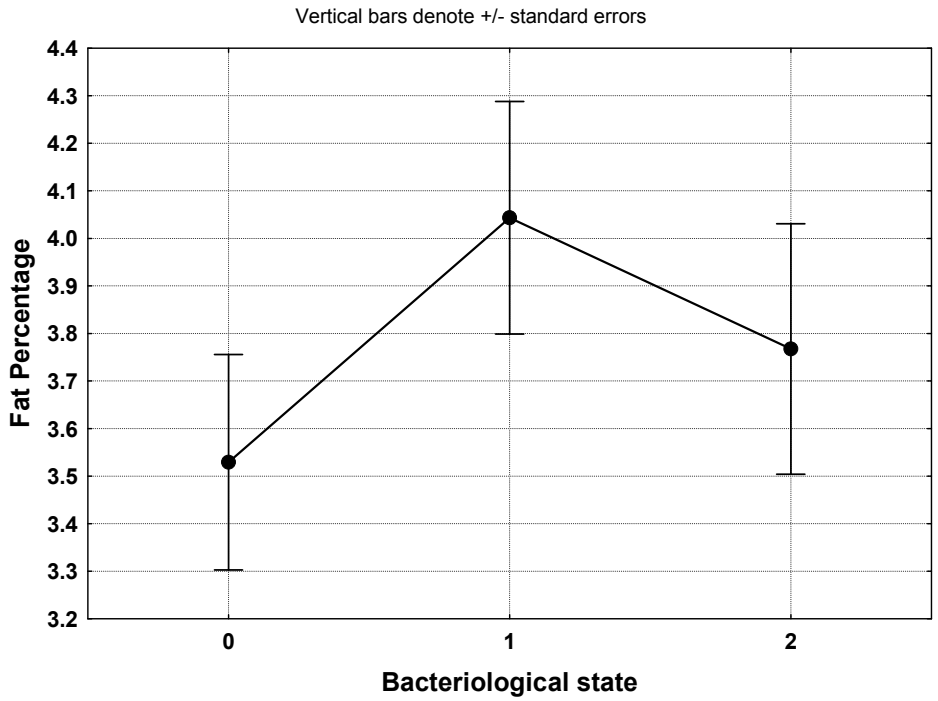
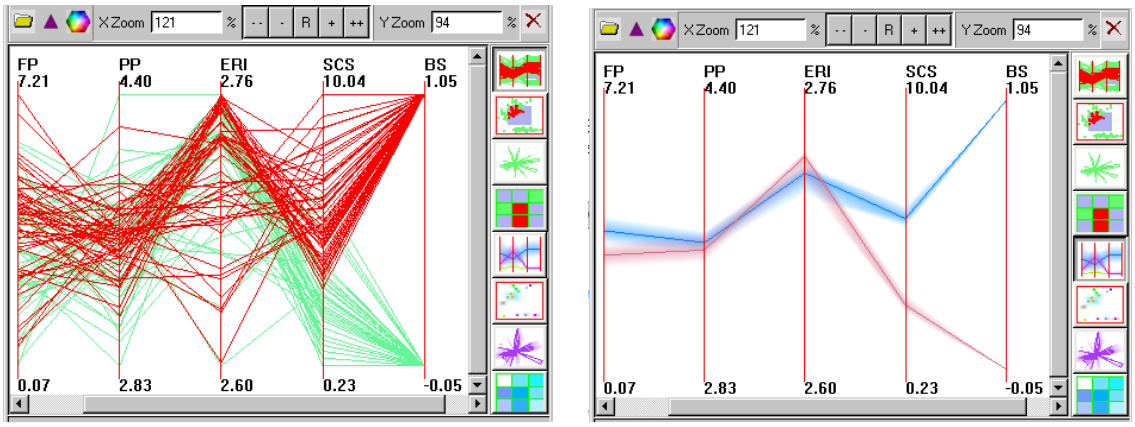


Figure 3.6.3.4 **Least square means difference of fat percentage between the three bacteriological states for training dataset (n=130).**

3.6.4 Multivariate Parallel Visualisation Analysis (MPVA)

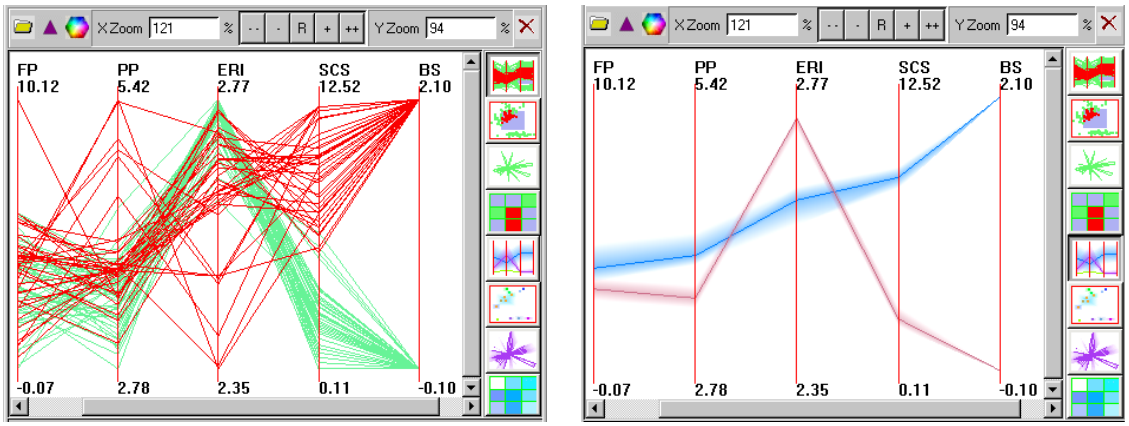
Results of the MPVA supported the correlation analysis. In general, as BS changed from NI to MNI (Figure 3.6.4.1 A and B), the mean values of SCS increased significantly, FP and PP increased slightly, while ERI decreased slightly (Figure: 3.6.4.1B). Here, again, SCS is observed as a major factor in discriminating between the two bacteriological states (NI vs. MNI). However, there were some overlapping regions, as some of the MNI cases had high ERI as well as high SCS (Figure 3.6.4.1 A), probably representing infection cases in their early stages.



A (Total Data Points) **B (Mean Values)**

BS = Bacteriological State [0=NI, 1=MNI]; SCS = Somatic Cell Score; ERI = Electrical Resistance Index; PP = Protein Percentage; FP = Fat Percentage.

Figure: 3.6.4.1 Multivariate parallel visualization of data: minor-infected and non-infected cases [Training dataset for unsupervised neural network model (n=130)].



A (Total Data Points) **B (Mean Values)**

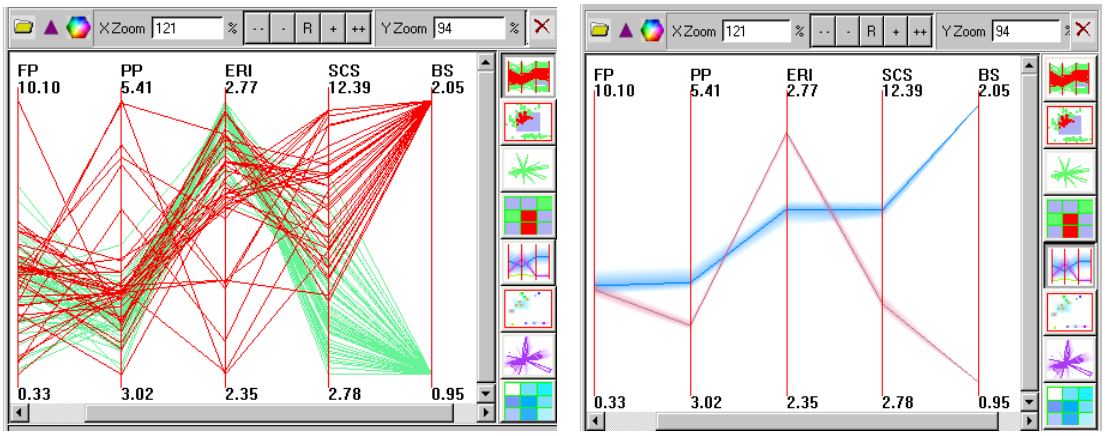
BS = Bacteriological State [0=NI, 2=MJI]; SCS = Somatic Cell Score; ERI = Electrical Resistance Index; PP = Protein Percentage; FP = Fat Percentage.

Figure 3.6.4.2 Multivariate parallel visualization of data: major-infected and non-infected cases [Training dataset for unsupervised neural network model (n=130)].

The same MPVA was also performed between the MJI and NI cases (Figure 3.6.4.2 A and B). Here the differences were more pronounced. For MJI

cases, mean values of SCS increased significantly, PP and FP slightly increased, and ERI significantly decreased.

The relationship between the MNI and MJI cases was also evaluated with the same MPVA method (Figure 3.6.4.3 A and B). This analysis indicated that the discrimination ability of SCS, ERI and PP was high, while FP was not good a discriminator. The mean values (Figure 3.6.4.3 B) of the milk parameters for the two bacteriological states were also significantly different except for FP.



A (Total Data Points) **B (Mean Values)**

BS = Bacteriological State [1=MNI, 2=MJI]; SCS = Somatic Cell Score; ERI = Electrical Resistance Index; PP = Protein Percentage; FP = Fat Percentage.

Figure 3.6.4.3 Multivariate parallel visualization of data: major-infected and minor-infected cases [Training dataset for unsupervised neural network model (n=130)].

The analyses indicate (Figures 3.6.4.1, 3.6.4.2 and 3.6.4.3) that SCS and ERI are the most informative milk parameters for the detection of infection status of a quarter. The SCS shows increased activity in the transition from non-infected to minor infected as well as major infected cases. The minor infections do not bring significant changes in the ERI and PP (Figure 3.6.3.1), while major infections strongly influence these two milk parameters (Figure 3.6.3.2). It was observed that FP has low potential for detecting the infection status of a quarter.

3.6.5 Principle Component Analysis (PCA)

Based on the correlation analysis, it was important to address the strong multicolinearity that existed between SCS, ERI, PP and FP. Therefore PCA was carried out and four principle components were extracted. The correlation matrix of variables was used to derive four PCs. The eigenvalues (EV), proportion of variance (PV) explained by each PC, cumulative eigenvalues (CE) and cumulative percentages (CP) of the variance of the four PCs were analysed.

PC-1 had the largest eigenvalue (1.54) and explained 38.6% of total variance in the data; followed by PC-2 (eigenvalue = 1.07; proportion of variance = 26.9%), PC-3 (eigenvalue = 0.9; proportion of variance = 22.5%) and PC-4 (eigenvalue = 0.47; proportion of variance = 11.8%). This analysis indicated that all the four PCs were important, because every PC explained a significant amount of variance in the data. The first three PCs explained 88% of variance in the data and therefore, were more important as compared to PC-4 which explained the remaining 11% of the variance.

The contributions of the original variables towards each PC were examined (Table 3.6.5.1). The SCS and ERI were strongly correlated and contributed most towards PC-1 (71%). The PP and ERI were also correlated and they contributed entirely towards PC-2 (100%). The FP which was not strongly correlated to SCS, ERI or PP, had maximum contribution towards PC-3 (84%), while PC-4 explained the remaining variance of SCS and ERI (77%).

Table 3.6.5.1 Factor/variable contributions (n=130).

Factor Variable Contributions				
	Factor 1	**Factor 2**	**Factor 3**	**Factor 4**
Somatic Cell Score	**0.46**	0.00	0.06	**0.47**
Electrical Resistance Index	**0.25**	**0.38**	0.07	**0.30**
Protein Percentage	0.13	**0.62**	0.02	0.22
Fat Percentage	0.15	0.00	**0.84**	0.00

The correlation of each variable with each PC was also examined (Table 3.6.5.2). The SCS (-0.85), PP (-0.45) and FP (-0.49) were negatively and ERI (0.62) positively correlated to PC-1. The ERI (-0.64) and PP (-0.82) were negatively correlated to PC-2. The FP (-0.87) was negatively correlated to PC-3. Finally, SCS (0.47) and ERI (0.38) were positively correlated to PC-4.

Table 3.6.5.2 Factor/variable correlations (n=130).

Factor Variable Correlations				
	Factor 1	**Factor 2**	**Factor 3**	**Factor 4**
Somatic Cell Score	**-0.85**	-0.05	0.24	**0.47**
Electrical Resistance Index	**0.62**	**-0.64**	-025	**0.38**
Protein Percentage	-0.45	**-0.82**	0.15	-033
Fat Percentage	-0.49	0.03	**-0.87**	-0.03

3.7 Development of Models

3.7.1 Introduction

In this study, unsupervised neural network and supervised neural network were the two modelling methodologies used for detecting mastitis causing major and minor bacterial pathogens present in milk samples. This section provides details of the configuration of the best models in terms of their correct classification rate for detecting the infection status of a quarter for the validation data sets. Sections 3.7.2 and 3.7.3 explore the configuration of unsupervised neural network and supervised neural network models, respectively.

3.7.2 Unsupervised Neural Network (USNN) Model Configuration

The data set mentioned in section 3.4 of this chapter was used for the development of the USNN model using Kohonen's self organising map algorithm (see Section 2.5.3 for details). The training data set was used to train a map with 66 neurons in the output layer of the model that clustered the input vectors. When the training error was reduced to a minimum level, the training was considered as complete. The trained neurons were further grouped into three clusters, representing the three bacteriological states using the SOM-Ward clustering method, which uses a combination of hierarchical clustering algorithm of Ward and SOM methods (Viscovery-SOMine, 2005).

The training data set containing the four principle components, days in milk ranges, lactation number, sire number, and bacteriological state were used to develop the USNN model. The model was validated using the validation data set with the four principle components, days in milk, lactation number and sire number.

3.7.3 Supervised Neural Network (SNN) Model Configuration

Supervised neural network learning paradigm was applied to develop SNN models using the datasets described in section 3.5. A variant of the back-propagation training algorithm known as quick-propagation was used in these

experiments (Fahlman, 1988). The algorithm treats the weights as if they were quasi-independent and attempts to use a simple quadratic model to approximate the error surface (Alyuda-Research, 2001-2005; Fahlman, 1988).

There were nine neurons in the input layer representing the input variables: four principle components, days in milk, lactation number and sire number (three neurons representing the three sire families). There were three neurons in the output layer representing the three bacteriological states.

Training and calibration datasets were used to train and calibrate the SNN models while the validation datasets were used to evaluate their generalization capability. Learning rate was kept at 0.1 for all experiments. Quick-propagation coefficient parameter of 1.75 was used. In the hidden and output layers, logistic activation function was used. The criterion for evaluating the models was based on their correct classification rate (CCR) on the validation data sets. Training was stopped when there was no improvement in the average CCR for the calibration data set.

3.7.4 Summary

This chapter explored the experimental data, data pre-processing steps and data partitioning steps before the application of advanced analytical methods. It is an established fact that using pre-processed data for the development of neural network models increases the probability of creating robust models. In contrast, raw data may contain outliers which may have adverse effects on the learning process of the neural network and thereby reduce the generalization capability of the model. The data analysis procedures and configurations of the neural network models were also explored in this chapter.

The following chapter provides details of the results of the neural network models on the validation data sets.

CHAPTER 4

4. RESULTS

4.1 Introduction

This chapter provides details of the results of the neural network models for the validation data sets. Section 4.2 explores the results for the USNN model, while section 4.3 for the SNN models.

4.2 Results of Unsupervised Neural Network Model

The USNN model was developed using the self organizing feature map algorithm. The configuration of the final trained and clustered neurons, representing the three bacteriological states is shown in the following Figure 4.2.1. The USNN model was able to identify the three bacteriological states using the milk parameters data. The neurons have been clustered in such a way that the neurons representing the non-infected state are distant from the neurons representing the major infected state, but border with the neurons representing the minor infected state.

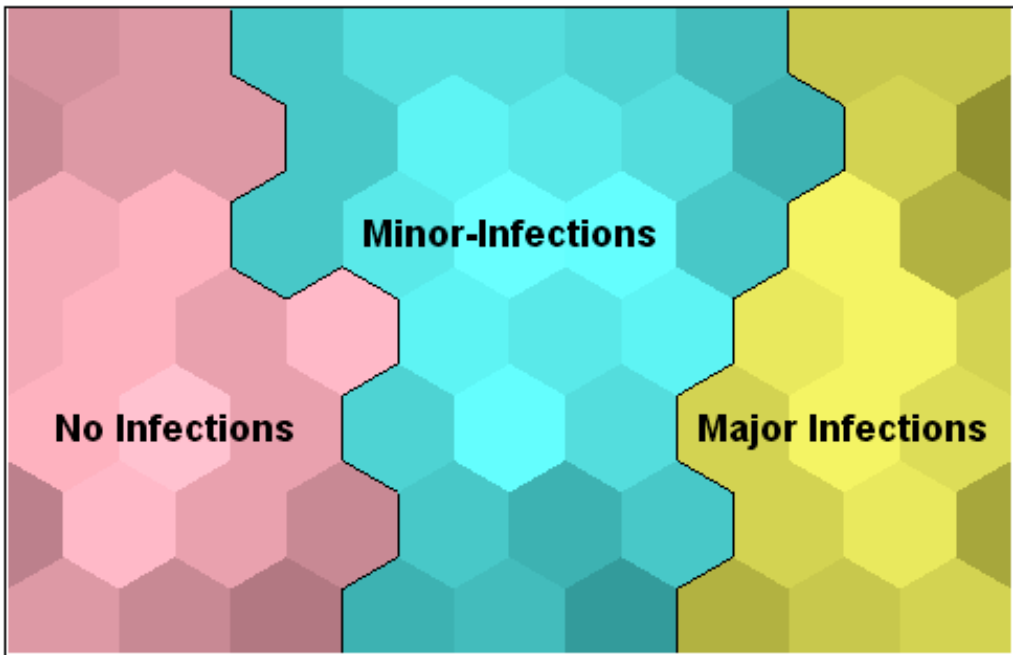


Figure 4.2.1 Clusters formed on the trained USNN model, representing three bacteriological states.

Figures 4.2.2 & 4.2.3 illustrate the values for PCs at each neuron of the trained map. The colors of the dots represent the values of a variable at that point in the map. The dark red color indicates a higher value, and the dark blue represents a lower value for a particular variable. The values of PC-1 decrease from non-infected towards minor and major infected cases, providing a good separation between the three infection states. PC-1 and PC-4 provided maximum discrimination between the three bacteriological states.

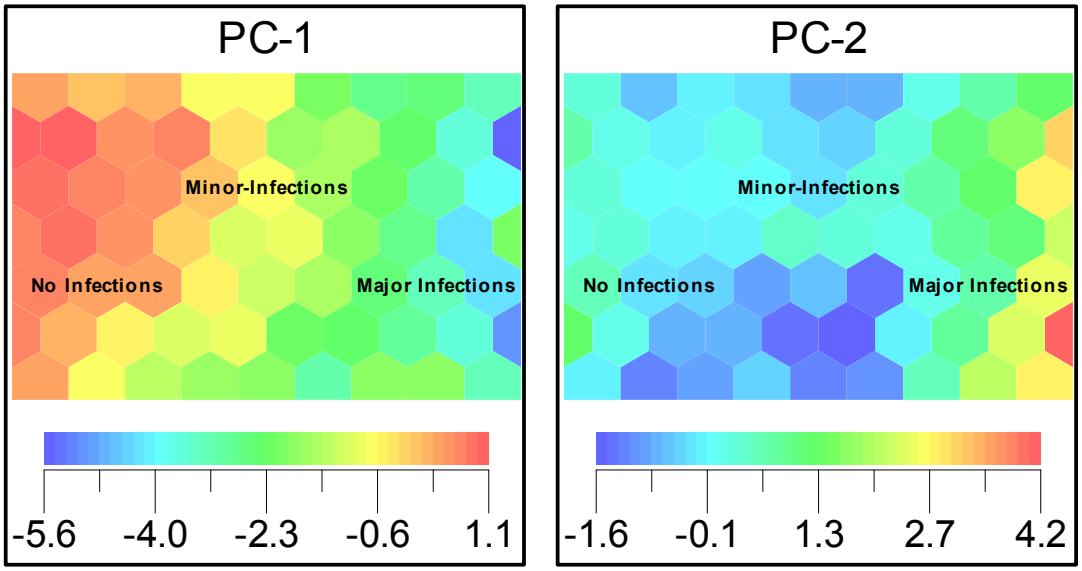


Figure 4.2.2 Representation of inputs PC-1 and PC-2 on the trained map.

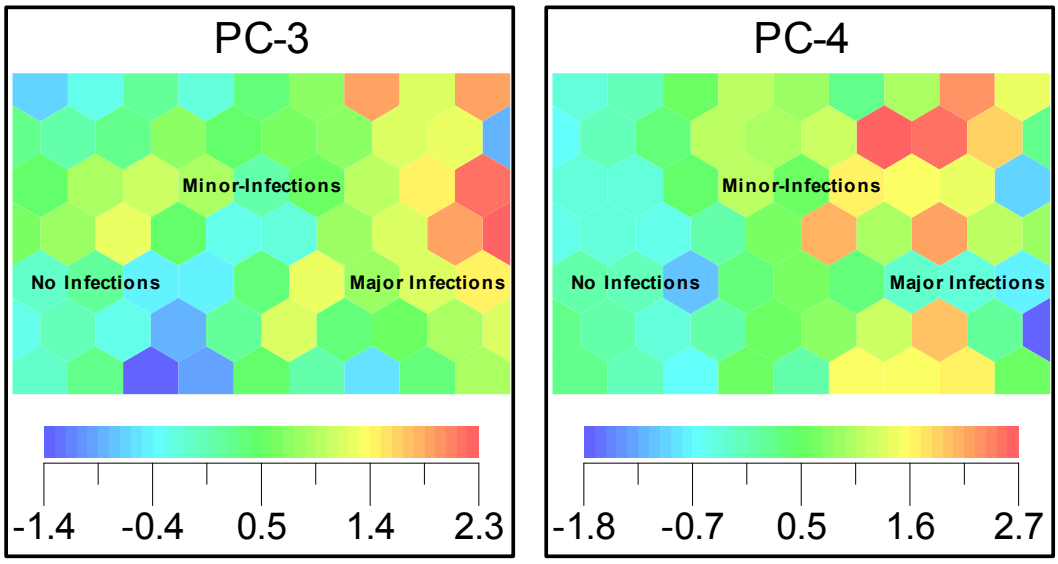


Figure 4.2.3 Representation of inputs PC-3 and PC-4 on the trained map.

The validation data set was used to assess the generalization ability of the model. The recall feature of the Viscovery® SOMine software was used to classify the validation data set using the trained and clustered map. As discussed earlier in section 3.7.2, only three clusters were formed based on the three infection states. The results are given in the following lines.

The classification results of the model for the validation dataset are shown in Table 4.2.1. Overall, the correct classification rate for this data set was 97%. Specificity of the model was 97% (4289/4411) and sensitivities for minor and major pathogens were 89% (269/301) and 80% (8/10), respectively. Misclassification rates were, 3% for non-infected cases (NI), 11% for minor infected (MNI) and 20% for the major infected (MJI) cases. A total of 1% (36/4411) NI cases were misclassified as MJI and 2% (86/4411) as MNI. Also, a total of 1% (4/301) of MNI cases were misclassified as NI and 9% (28/301) as MJI. Similarly, 20% (2/8) of MJI cases were misclassified as MNI and none as NI.

Table 4.2.1 Matching matrix of observed and predicted classes using the

unsupervised neural network model (validation data set n=4722).

Bacteriological status	Observed (n)	USNN Predicted (n)				Specificity and Sensitivity
		NI	MNI	MJI	Total	(%)
Not Infected (NI)	4411	4289	86	36	4411	**97**
Minor Infected (MNI)	301	4	269	28	301	**89**
Major Infected (MJI)	10	0	2	8	10	**80**
Total	**4722**				**4722**	

In terms of mastitis pathogens, 87% of *coagulase-negative staphylococci* (131/150) and 92% of *Corynebacterium bovis* (138/150) were classified correctly in the MNI category. For MJI, 71% of *Streptococcus uberis* (5/7), 100% of *Streptococcus dysgalactiae* (2/2) and 100% of *Streptococcus agalactiae* (1/1) were classified correctly.

Differences in mean values of the milk parameters and PCs were analysed (Table 4.2.2) for each cluster using One Way ANOVA. Mean values were different ($p < 0.001$) for milk parameters as well as each PC, with the exception of PP. Principle components provided maximum discrimination between each bacteriological state.

Table 4.2.2 Milk parameter means (±SEM) for each bacteriological status

cluster formed on the self organizing map for the validation dataset. Milk samples were clustered into not-infected (NI), infected by minor pathogens (MNI) or infected by major pathogens (MJI).

	Bacteriological Status Cluster			
Milk Parameters	**NI**	**MNI**	**MJI**	**($p < 0.001$)**
Somatic cell score	3.14 ± 0.02	5.31 ± 0.07	7.06 ± 0.15	All clusters
Electrical resistance index	0.72 ± 0.00	0.71 ± 0.00	0.57 ± 0.00	MJI
Milk protein (%)	3.57 ± 0.00	3.56 ± 0.01	3.57 ± 0.03	Not Significant
Milk fat (%)	3.44 ± 0.02	4.03 ± 0.07	2.88 ± 0.17	All clusters
Principle component-1	0.15 ±0.01	-0.76 ± 0.04	-2.66 ± 0.09	All clusters
Principle component-2	-0.03 ± 0.01	-0.31 ± 0.05	2.46 ± 0.11	All clusters
Principle component-3	-0.08 ± 0.02	0.43 ± 0.05	1.61 ± 0.11	All clusters
Principle component-4	-0.08 ± 0.01	0.88 ± 0.05	-0.49 ± 0.11	All clusters

4.3 Results of Supervised Neural Network Models

This section provides details of the supervised neural network modelling experiments for the four datasets developed in Section 3.5. The inputs used in these experiments were the four principle components, days in milk, lactation number and sire number. The output was the bacteriological status of each quarter.

The heuristic network architecture search method of the Alyuda NeuroIntelligence 2.2 software (Alyuda-Research, 2001-2005; Fahlman, 1988) was used to search for the best network. A range from 2 to 200 neurons in the hidden layer was used to search for the optimal number of neurons in the hidden layer.

4.3.1 Results of Supervised Neural Network (SNN) Models for Dataset 1 (Infected to non-infected ratio 1:1)

Table 4.3.1.1 provides details of the results of the trained SNN models using the first dataset described in Section 3.5. Models number 7, 8, 12, 13 and 14 with hidden neurons ranging between 87 and 97, achieved highest accuracy for this data set. The CCR on the validation datasets of these models ranged from 76% to 80%. The effect of the different number of neurons in the hidden layer on the correct classification rate (CCR) of the model was evident. When the number of neurons in the hidden layer was either increased from 97 or decreased from 87, the average CCR deteriorated.

Table 4.3.1.1 Results of models trained using dataset with 1:1 ratio of

infected to non-infected cases.

Model Number	Multi Layer Perceptron Network Architecture			Number of Weights	Overall Correct Classification Rate (CCR) %		
	Input Layer	Hidden Layer	Output Layer		Training Set	Calibration Set	Validation Set
1	9	2	3	29	77	78	71
2	9	200	3	2603	84	83	74
3	9	124	3	1615	86	86	74
4	9	77	3	1004	95	84	74
5	9	48	3	627	95	85	72
6	9	106	3	1381	89	84	72
7	9	94	3	1225	90	85	79
8	9	87	3	1134	98	85	76
9	9	101	3	1316	84	84	75
10	9	98	3	1277	83	86	70
11	9	91	3	1186	87	87	73
12	9	96	3	1251	93	84	77
13	**9**	**97**	**3**	**1264**	**94**	**85**	**80**
14	9	95	3	1238	93	84	76

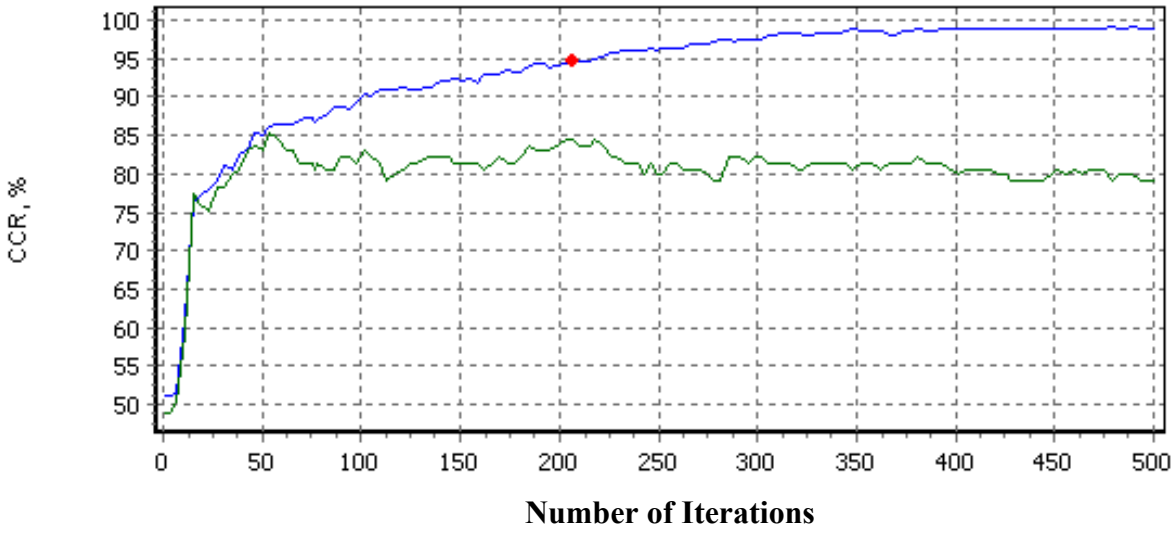


Figure 4.3.1.1 Correct classification rate (vertical axis) for training (blue line) and calibration (green Line) datasets for the best model against number of training iterations; dataset with 1:1 ratio of infected to non-infected cases.

The best network was number 13 with 97 neurons in the hidden layer. For this network, Figure 4.3.1.1 shows the changes in CCR as the training

progressed. The blue line indicates the CCR for the training dataset while the green line for the calibration dataset. The network was saved at a point (seen as a red dot) where the classification accuracy was high for the calibration dataset. Beyond this point it was found that the network was learning noise or peculiarities in the training dataset without any improvement on the calibration dataset.

The model (# 13) was evaluated using the validation data set. The overall CCR for validation data set was 80%. The specificity of the model for correctly detecting non-infected cases was 82%, while, sensitivities for correctly detecting infected cases were 86% for minor infections and 20% for major infections (Table 4.3.1.2). Due to data limitations, the number of major infected category in the training data set was low, resulting in a low CCR for this category.

Table 4.3.1.2 Matching matrix of observed and predicted classes from the supervised neural network model based on the validation dataset with infected to non-infected cases ratio of 1:1.

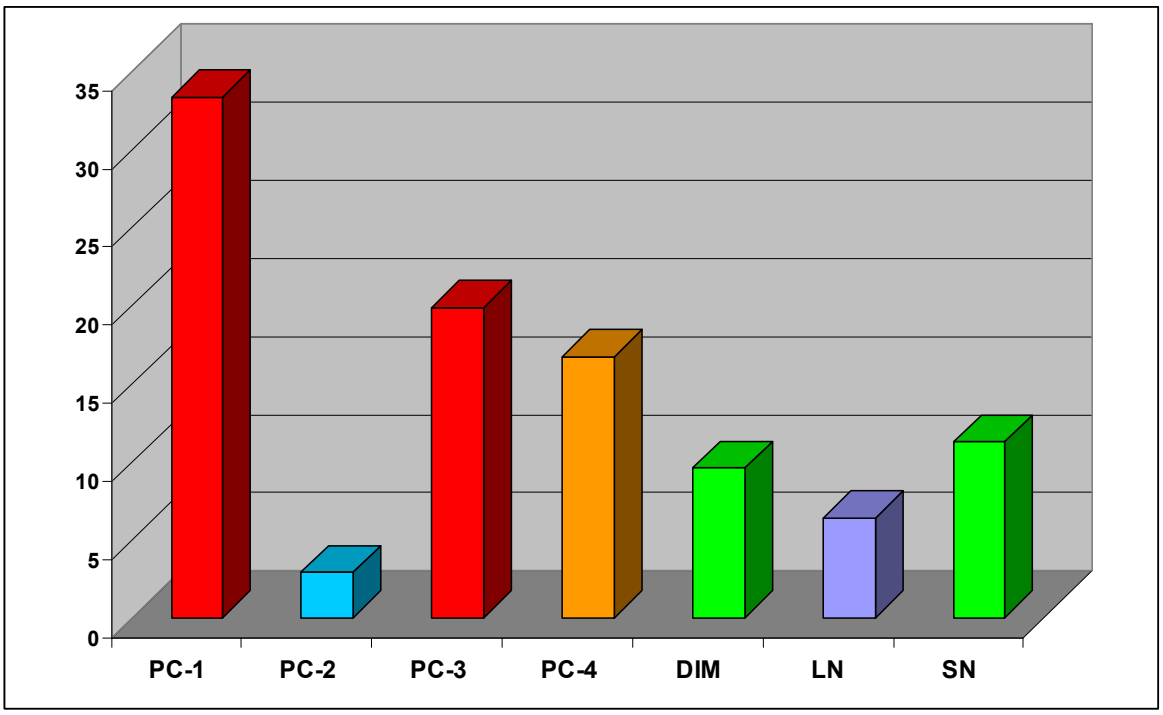
Bacteriological status	Observed (n)	SNN Predicted (n)				Specificity and Sensitivity
		NI	MNI	MJI	Total	(%)
Not Infected (NI)	82	67	15	0	82	**82**
Minor Infected (MNI)	71	10	61	0	71	**86**
Major Infected (MJI)	10	0	8	2	10	**20**
Total	**163**				**163**	

The impact of each input variable on the model output was evaluated (Table 4.3.1.3) using sensitivity analysis. This analysis was performed by removing one input variable at a time and analysing the deterioration in the model output. It was found that PC-1, PC-3 and PC-4, representing SCS, ERI and FP were the

most important input variables for this model. Figure 4.3.1.2 provides the results of sensitivity analysis in graphical format.

Table 4.3.1.3 Results of sensitivity analysis of the model, contribution of each input variable towards the model output for the dataset with infected to non-infected cases ratio of 1:1.

with Input Variables	Contribution, %
Principle Component – 1	**33**
Principle Component – 2	**3**
Principle Component – 3	**19**
Principle Component – 4	**16**
Days In Milk	**9**
Lactation Number	**6**
Sire Number	**11**



Key: PC-1 = Principle Component 1, PC-2 = Principle Component 2, PC-3 = Principle Component 3, PC-4 = Principle Component 4, DIM = Days in Milk, LN = Lactation Number, SN = Sire Number.

Figure 4.3.1.2 Results of sensitivity analysis in graphical format. On the horizontal axis are input variables and on the vertical axis their contribution to the model output based on the validation dataset with infected to non-infected cases ratio of 1:1.

4.3.2 Results of Supervised Neural Network Models for Dataset 2 (Infected to non-infected ratio 1:2)

Table 4.3.2.1 provides details of the results of the trained models using the second dataset as described in Section 3.5. Models number 4, 6, 9, 13 and 14 with hidden neurons ranging between 77 and 118, achieved highest accuracy for this dataset (ratio 1:2 of infected to non-infected cases). The overall CCR on the validation datasets of these models ranged from 82% to 84%. The changing number of neurons in the hidden layer affected the CCR of the networks; when the number of neurons in the hidden layer was either increased from 118 or decreased from 77, the average CCR deteriorated.

The best network was number 9 with 112 neurons in the hidden layer. Figure 4.3.2.1 shows the changes in CCR for this network as the training progressed. The blue line indicates the CCR for the training dataset while the green line for the calibration dataset. The network was saved at a point (seen as a red dot) where the classification accuracy was the highest for the calibration dataset. Beyond this point, the performance of the network improved for the training dataset without any improvement on the calibration dataset.

Table 4.3.2.1 Results of the models trained using the dataset with 1:2 ratio of infected to non-infected cases.

Model Number	Multi Layer Perceptron Network Architecture			Number of Weights	Overall Correct Classification Rate (CCR) %		
	Input Layer	Hidden Layer	Output Layer		Training Set	Calibration Set	Validation Set
1	9	2	3	29	84	85	79
2	9	200	3	2603	86	88	80
3	9	124	3	1615	87	86	81
4	9	77	3	1004	87	84	82
5	9	48	3	627	88	85	79
6	9	106	3	1381	86	86	82
7	9	94	3	1225	87	86	80
8	9	117	3	1524	91	85	81
9	**9**	**112**	**3**	**1459**	**91**	**85**	**84**
10	9	121	3	1576	90	86	79
11	9	115	3	1498	87	84	80
12	9	119	3	1550	87	86	79
13	9	118	3	1537	90	86	83
14	9	116	3	1511	93	85	83

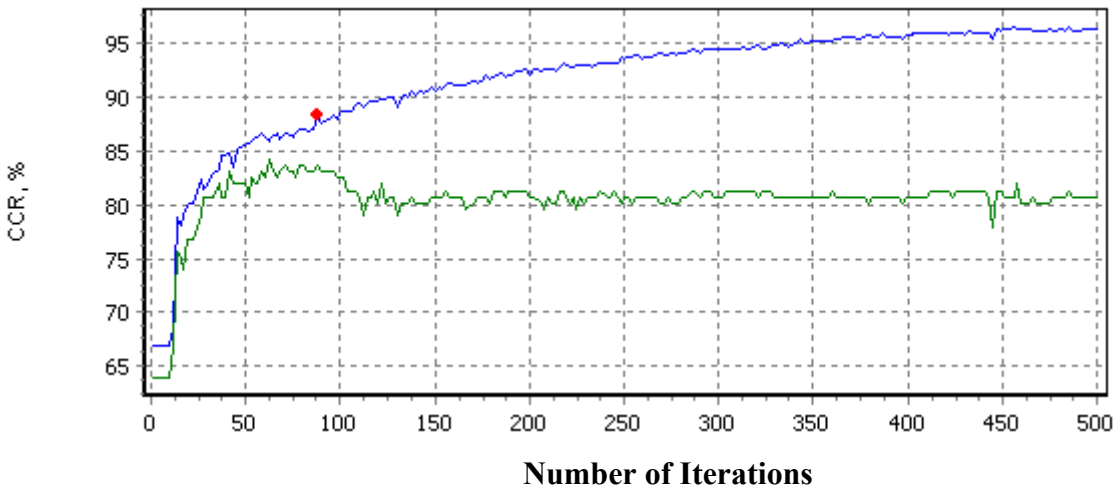


Figure 4.3.2.1 Correct classification rate (vertical axis) for training (blue line) and calibration (green Line) datasets for the best model; [ratio of infected to non-infected cases of 1:2].

The model was evaluated using the validation data set. The overall CCR for validation data set was 84%. The specificity of the model for correctly detecting non-infected cases was 91%, while, sensitivities for correctly detecting infected cases were 76% for minor infections and 20% for major infections (Table 4.3.2.2).

These results reveal the effect of increasing the proportion of non-infected cases. For example, there is a substantial increase in the correct classification of these healthy cases from 82% to 91%. Adding healthy cases has also increased the region of overlap between healthy and minor infection regions thereby reducing correct classification of minor infections. It has also pushed the minor and major infection regions closer thereby classifying some minor infections as major infections.

Table 4.3.2.2 Matching matrix of observed and predicted classes from the supervised neural network model based on validation data set with infected to non-infected cases ratio of 1:2.

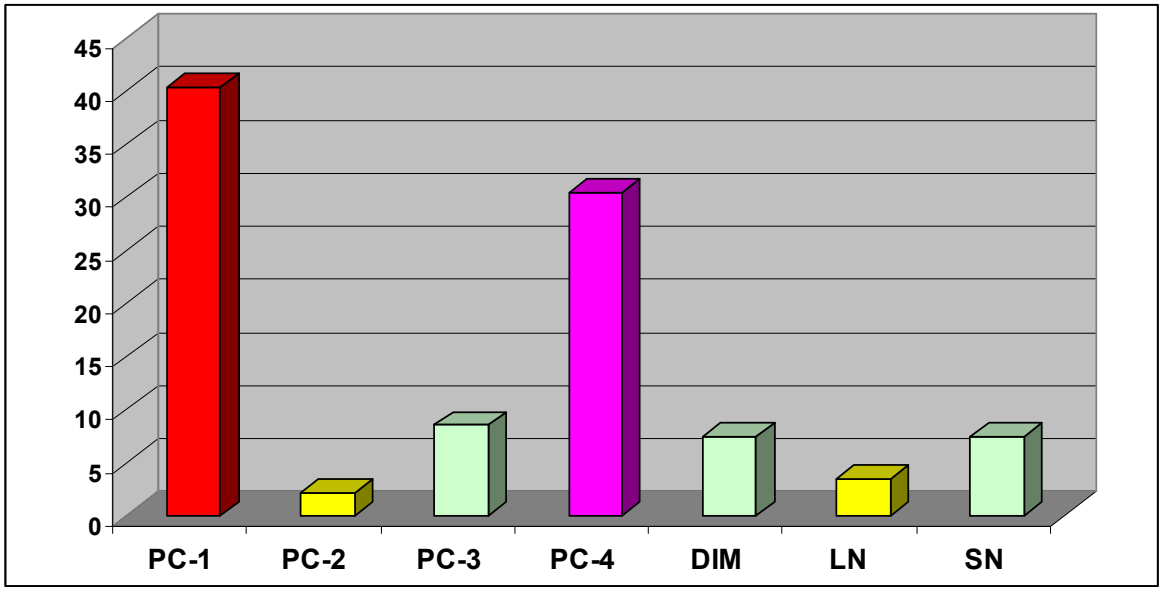
Bacteriological status	Observed (n)	SNN Predicted (n)				Specificity and Sensitivity
		NI	MNI	MJI	Total	(%)
Not Infected (NI)	170	154	16	0	170	**91**
Minor Infected (MNI)	71	14	54	3	71	**76**
Major Infected (MJI)	10	0	8	2	10	**20**
Total	**251**				**251**	

The impact of each input variable on the model output was evaluated (Table 4.3.2.3) using sensitivity analysis. It was found that PC-1 and PC-4, representing SCS and ERI, were the most important input variables for this model.

Compared to the case with 1:1 ratio of infected to non-infected cases, these results suggest that the effect of the minor variable Fat percentage, is masked when the prevalence of non-infected cases increases. Also, the relevance of other variables DIM and Sire number has slightly diminished. Figure 4.3.2.2 provides the results of sensitivity analysis in graphical format for this model.

Table 4.3.2.3 Contribution of each input variable to the model output of the best network based on sensitivity analysis for the dataset with infected to non-infected cases ratio of 1:2.

Input Variables	Contribution, %
Principle Component –1	**45**
Principle Component –2	**2**
Principle Component –3	**6**
Principle Component –4	**22**
Days In Milk	**5**
Lactation Number	**9**
Sire Number	**9**



Key: PC-1 = Principle Component 1, PC-2 = Principle Component 2, PC-3 = Principle Component 3, PC-4 = Principle Component 4, DIM = Days in Milk, LN = Lactation Number, SN = Sire Number.

Figure 4.3.2.2 Results of sensitivity analysis in graphical format. On the horizontal axis are input variables and on the vertical axis are their percentage contributions, assessed on the dataset with infected to non-infected cases ratio of 1:2.

4.3.3 Results of Supervised Neural Network Models for Dataset 3 (ratio of infected to non-infected cases of 1:4)

Table 4.3.3.1 provides details of the results of the trained models using the third dataset described in Section 3.5. Models number 3, 4, 7, 9, 13 and 15 with hidden neurons ranging from 77 to 124, achieved highest accuracy for this dataset. The overall CCR on the validation dataset of these models ranged from 84% to 86%.

Table 4.3.3.1 Results of models trained using dataset with 1:4 ratio of infected to non-infected cases.

Model Number	Multi Layer Perceptron Network Architecture			Number of Weights	Overall Correct Classification Rate (CCR) %		
	Input Layer	Hidden Layer	Output Layer		Training Set	Calibration Set	Validation Set
1	9	2	3	29	86	84	82
2	9	200	3	2603	90	87	83
3	9	124	3	1615	90	87	85
4	9	77	3	1004	91	87	85
5	9	48	3	627	91	87	82
6	9	106	3	1381	93	86	83
7	**9**	**94**	**3**	**1225**	**91**	**88**	**86**
8	9	65	3	848	94	88	82
9	9	87	3	1134	92	87	84
10	9	72	3	939	91	89	81
11	9	83	3	1082	92	88	82
12	9	80	3	1043	92	89	83
13	9	75	3	978	90	88	84
14	9	78	3	1017	91	88	82
15	9	76	3	991	91	86	84

The best network was number 7 with 94 neurons in the hidden layer. Figure 4.3.3.1 shows the changes in the overall CCR for this network as the training progressed. The blue line indicates the CCR for the training data set while the green line indicates that for the calibration data set. The network was saved at a point (seen as a red dot) where the classification accuracy was the

highest for the calibration data set. Training the model beyond this point did not improve its performance on the calibration data set.

Figure 4.3.3.1 Correct classification rate (Y-axis) for training (blue line) and calibration (green Line) datasets for the best model against training iterations for the dataset with infected to non-infected cases ratio of 1:4.

Model was evaluated using the validation dataset. The overall CCR for validation dataset was 86%. The specificity of the model for correctly detecting non-infected cases was 94%, while, sensitivities for correctly detecting infected cases were 71% for minor infections and 30% for major infections (Table 4.3.3.2). Compared to the previous model (with dataset 1:2 ratio), CCR for the non-infected and major infected cases increased while for minor infected cases a decrease was observed.

Here, the effect of adding more NI cases is to further improve classification accuracy of NI cases and to continue the trend of deteriorating classification accuracy on MNI further taking it down to 71% compared to 76% for 1:2 ratio and 86% for 1:1 ratio. There is a 10% increase in the classification accuracy of MJI, although, this is due to classifying one more case accurately.

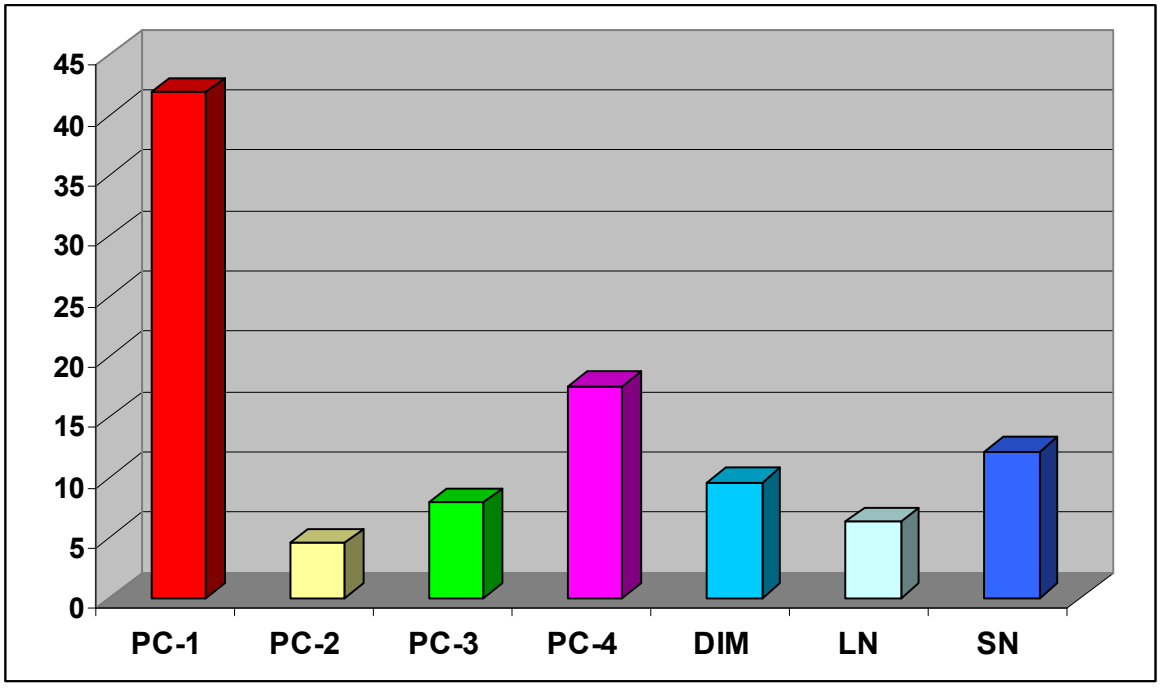
Table 4.3.3.2 Matching matrix of observed and predicted classes from the supervised neural network model based on the validation dataset with infected to non-infected cases ratio of 1:4.

Bacteriological status	Observed (n)	SNN Predicted (n)				Specificity and Sensitivity
		NI	MNI	MJI	Total	(%)
Not Infected (NI)	313	295	18	0	313	**94**
Minor Infected (MNI)	71	26	42	3	71	**59**
Major Infected (MJI)	10	0	7	3	10	**30**
Total	**394**				**394**	

The impact of each input variable on the model output was evaluated (Table 4.3.3.3) using sensitivity analysis. It was found that PC-1 and PC-4, representing SCS and ERI, were the most important input variables for this model. The contribution of PC-1 was 42% and that of PC-2 was 18% followed by days in milk and sire number. These results are presented in graphical format in Figure 4.3.3.2.

Table 4.3.3.3 Contribution of input variables to the output of the network based on sensitivity analysis [dataset with infected to non-infected cases ratio of 1:4].

Input Variables	Contribution, %
Principle Component –1	**42**
Principle Component –2	**5**
Principle Component –3	**8**
Principle Component –4	**18**
Days In Milk	**10**
Lactation Number	**6**
Sire Number	**12**



Key: PC-1 = Principle Component 1, PC-2 = Principle Component 2, PC-3 = Principle Component 3, PC-4 = Principle Component 4, DIM = Days in Milk, LN = Lactation Number, SN = Sire Number.

Figure 4.3.3.2 Results of sensitivity analysis in graphical form. On the horizontal axis are input variables and on the vertical axis is their percentage contributions based on the dataset with infected to non-infected cases ratio of 1:4.

4.3.4 Results of Supervised Neural Network Models for Dataset 4 with infected to non-infected cases ratio of 1:10

Table 4.3.4.1 provides details of the results of the trained models using the fourth dataset as described in Section 3.5. Models number 5, 7, 10, 11, 12 and 13 with hidden neurons ranging from 167 to 188, achieved highest accuracy for this data set.

Table 4.3.4.1 Results of models trained using the dataset with 1:10 ratio of infected to non-infected cases.

Model Number	Multi Layer Perceptron Network Architecture			Number of Weights	Overall Correct Classification Rate (CCR) %		
	Input Layer	Hidden Layer	Output Layer		Training Set	Calibration Set	Validation Set
1	9	2	3	29	92	92	90
2	9	200	3	2603	96	94	91
3	9	124	3	1615	95	94	91
4	9	77	3	1004	96	94	91
5	9	170	3	2213	94	94	92
6	9	152	3	1979	96	94	91
7	**9**	**188**	**3**	**2447**	**94**	**93**	**93**
8	9	181	3	2356	96	94	91
9	9	163	3	2122	96	94	91
10	9	176	3	2291	94	94	92
11	9	167	3	2174	96	94	92
12	9	173	3	2252	94	94	92
13	9	171	3	2226	96	94	92
14	9	168	3	2187	96	93	91
15	9	169	3	2200	94	94	91

The best network was number 7 with 188 neurons in the hidden layer. For this network Figure 4.3.4.1 shows the changes in overall CCR as the training progressed. The blue line indicates the CCR for the training data set while the green line for the calibration data set. The network was saved at a point (seen as a red dot) where the classification accuracy was the highest for the calibration data

set; further training beyond this point was not helpful in improving the performance of the model on the calibration data set.

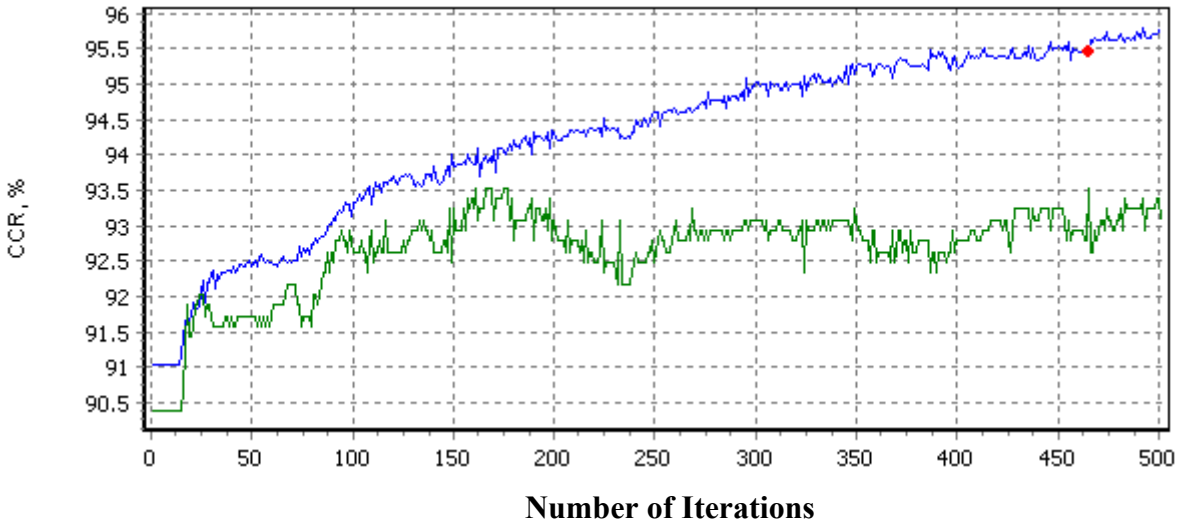


Figure 4.3.4.1 Overall correct classification rate for training (blue line) and calibration (green Line) datasets for the best model against training iterations; dataset with 1:10 ratio of infected to non-infected cases.

The best model (# 7) was evaluated using the validation dataset. The overall CCR for validation dataset was 93%. The specificity of the model for correctly detecting non-infected cases was 98%, while, sensitivities for correctly detecting infected cases were 44% for minor infections and 40% for major infections (Table 4.3.4.2).

Table 4.3.4.2 Matching matrix of observed and predicted classes using the supervised neural network model based on the validation data set with infected to non-infected cases ratio of 1:10.

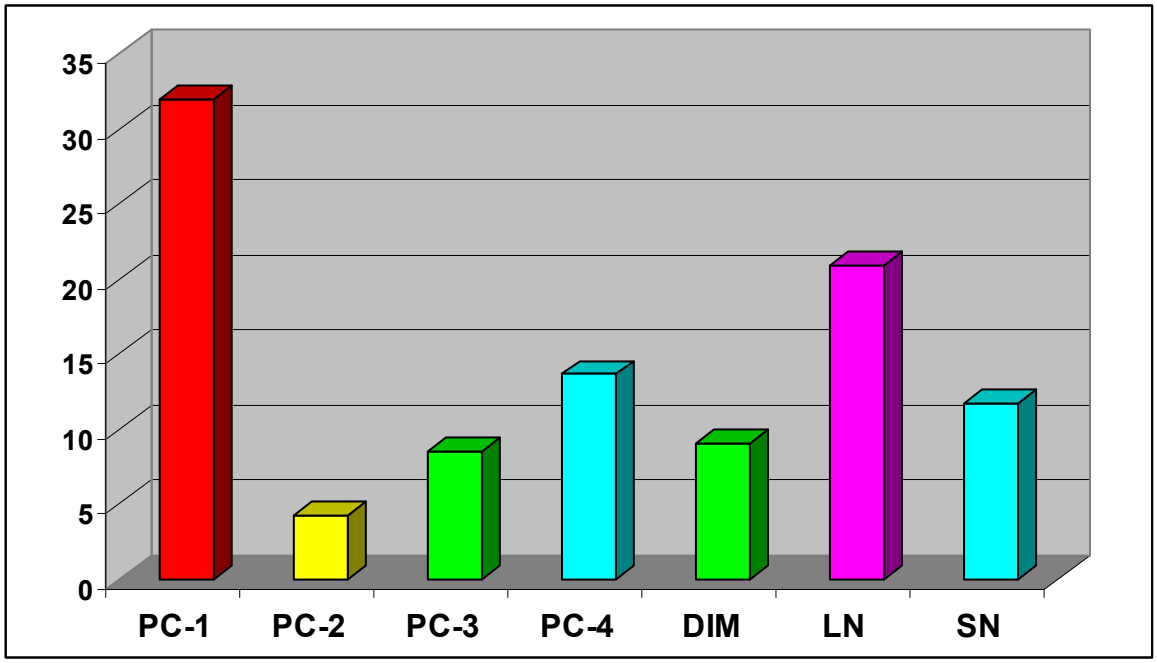
Bacteriological status	Observed (n)	SNN Predicted (n)				Specificity and Sensitivity
		NI	MNI	MJI	Total	(%)
Not Infected (NI)	780	763	17	0	780	**98**
Minor Infected (MNI)	71	38	31	2	71	**44**
Major Infected (MJI)	10	1	5	4	10	**40**
Total	**861**				**861**	

As expected, the classification rate for NI cases increased to 98% due to the increase of NI cases in the dataset. Continuing the previous trend, the CCR for MNI plummeted to 44%. However, CCR of MJI increased from 30% to 40%, although again this was the result of classifying one new case correctly. Nevertheless, it could be a realistic trend due to sharpening of boundary of the MJI region with the increase of NI cases. However, this minor increase in classification rate for NI and MJI cases comes at a great expense of misclassifying MNI. The use of realistic infected to non-infected ratio in the dataset appeared to misclassify a large quantity of MNI. It may be advisable to use realistic ratios for effectively discriminating between NI and MJI cases, and an artificially reduced ratio for separating MNI from NI cases.

The impact of each input variable on the model output was evaluated (Table 4.3.4.3) using sensitivity analysis. It was found that PC-1, PC-4, lactation number and sire number were the most important input variables for this model. As compared to the data set with infected to non-infected ratio of 1:4, the contribution of PC-1and PC-4 decreased while that of lactation number increased. For PC-2, PC-3, DIM and Sire number no substantial change was observed. These results in graphical format are presented in Figure 4.3.4.2.

Table 4.3.4.3 Contribution of input variables to the output of the network based on sensitivity analysis on the dataset with infected to non-infected cases ratio of 1:10.

Input Variables	Contribution, %
Principle Component -1	**32**
Principle Component -2	**4**
Principle Component -3	**8**
Principle Component -4	**14**
Days In Milk	**9**
Lactation Number	**20**
Sire Number	**12**



Key: PC-1 = Principle Component 1, PC-2 = Principle Component 2, PC-3 = Principle Component 3, PC-4 = Principle Component 4, DIM = Days in Milk, LN = Lactation Number, SN = Sire Number.

Figure 4.3.4.2 Results of sensitivity analysis in graphical form. On the horizontal axis are input variables and on the vertical axis is their contribution (dataset with infected to non- infected cases ratio of 1:10).

4.4 Summary

This section explored the results of the USNN and SNN modelling experiments in detecting the infection status of a quarter. Training datasets were used to develop the models and the validations datasets were used to assess their generalisation capability.

The USNN model achieved a specificity (correctly identifying non-infected cases) of 97% and sensitivities for correctly identifying minor and major infected cases were 89% and 80%, respectively. Analysis of the clusters of three infection states revealed that all four PCs were significantly different for each cluster. However, PC-1 and PC-4 representing SCS and ERI were found to be the most important input variables for this model.

The overall CCR of the SNN ranged from 80 to 93%. The CCR for the NI cases ranged from 82 to 98%, while for minor and major infected cases ranged from 44 to 86% and 20 to 40%, respectively. Increasing the proportion of NI cases in the data set improved the classification accuracy for NI and MJI cases; however, this increase came at the cost of misclassifying more of the MNI cases.

Results of the sensitivity analysis for the SNN suggested that PC-1, PC-3 and PC-4, representing SCS, ERI and FP were the most important input variables. These results were consistent with those of the USNN model, where SCS and ERI were found to be the most important variables. The next section provides a detailed discussion on the results of this research.

CHAPTER 5

5. DISCUSSION AND CONCLUSIONS

5.1 General Discussion

Mastitis is the most costly disease for the dairy industry around the world. This disease is caused by minor and major bacterial pathogens. The main objectives of this research were:

1. To identify the most informative milk parameters for the identification of minor and major bacterial pathogens;

2. To develop and evaluate supervised and unsupervised neural network models for the detection of these pathogens in the early stages of the disease;

4. Evaluate the impact of different proportions of infected to non-infected cases on the correct classification rate of the supervised neural network models as there are proportionately less infected cases in a herd compared to non-infected cases.

To achieve the above objectives, a database was used that contained quarter based milk parameters and bacteriological examination data.

To achieve the first objective of this research, correlation analysis, analysis of variance and multivariate parallel visualisation analysis were used. The results of these analyses are discussed in the following paragraphs.

It is well known that SCC/SCS reflects the immune response of a cow to bacterial infections, which was also observed in this study. For example, SCS shows the highest increase as a response to minor infections compared to small changes in the other milk parameters (Table 3.6.3.1, Figures 3.6.3.1, 3.6.3.2,

3.6.3.3, 3.6.3.4, 3.6.4.1). The high correlation between SCS and bacteriological state observed in this study (Table 3.6.2.1) agrees with Berning and Shook (1992), although they found that SCS did not differentiate between the MNI and MJI cases. In the current study, it was found that SCS can effectively discriminate between NI, MNI and MJI cases (Figures 3.6.3.1, 3.6.4.1, 3.6.4.2 and 3.6.4.3; Table 3.6.3.1).

Another useful milk parameter for discriminating between infected and non-infected cases was electrical resistance (ER) [inverse of electrical conductivity (EC)], and more specifically electrical resistance index (ERI). The EC/ER is the reflection of ionic changes resulting from the damage caused by bacteria to the alveoli (Kitchen *et al.*, 1980). It was found that ERI was lowest for MJI cases, suggesting that tissue damage caused by major pathogens was noticeably higher compared to minor pathogens (Figures: 3.6.3.2, 3.6.4.1, 3.6.4.2 & 3.6.4.3; Table: 3.6.3.1). The mean difference in the ERI values between the NI and MNI cases was very low, suggesting little damage of mammary tissues by the minor pathogens. This indicates that ERI has greater ability in discriminating glands infected with major pathogens from glands that are not infected or that have a minor pathogen infection.

Mastitis-related changes in protein content are not conclusive as results reported in literature are either contradictory or at odds. Mastitis may cause milk protein content to increase (Auldist, Coats, Rogers, & McDowell, 1995), decrease (Lee, Yu, Jeong, Back, & Yoon, 1991) or even stay unchanged (Rogers, Mitchell, & Bartley, 1989). In the current study, it was found that there was no significant difference in the protein percentage (PP) of NI and MNI cases (Table: 3.6.3.1), which agrees with the findings of Rogers *et al*. (1989). However, PP was higher for MJI cases (Table: 3.6.3.1; Figures: 3.6.3.3, 3.6.4.2 and 3.6.4.3), which agrees with Auldist *et al.* (1995).

In a similar manner, mastitis-related changes in fat percentage (FP) are also not conclusive. Mastitis causes changes in the milk fat content, but without a definitive trend, as it may increase (Mitchell, Rogers, Houlihan, Tucker, & Kitchen, 1986), decrease (Kitchen, 1981) or remain unchanged (Rogers *et al.*, 1989). In this study, no significant change in FP was observed between NI, MNI and MJI cases (Table: 3.6.3.1; Figures: 3.6.3.4, 3.6.4.1, 3.6.4.2, 3.6.4.3), which agrees with Rogers *et al.* (1989).

To achieve the second objective of this research, unsupervised and supervised neural network models were developed. The USNN model classified the validation data set with an overall CCR of 97% (Table: 4.2.1). These results are superior to previous models developed by Heald *et al*. (2000), where the overall correct classification rate ranged from 57% to 71%. The input variables and neural network learning paradigm used by Heald *et al*. (2000) were different to those used in the current study. They classified pathogens according to their mode of transmission (contagious, environmental & others). In the current study, cases were classified based on the response of the mammary gland to infection, which gave better results.

In some instances the USNN model classed cases into a wrong cluster. For example few NI cases were classed as MJI by the model probably because they had high SCS and low ERI values. Similarly, some NI cases were also classed as MNI due to their relatively high SCS and low ERI values. One reason for these results could be that these were either cases of early or late infections which were not detected during the bacteriological examinations. Another reason could be that the measurement devices failed to give correct readings for the milk parameters for these cases. In addition to the above two possible reasons, these few cows may have had inherently high SCS and low ERI values, making it difficult for the model to detect them correctly.

The overall CCR of the SNN models (SNN) ranged from 80-93%. The CCR of the SNN models was low compared to the USNN model developed in the current study. However, results of the current SNN modelling experiments were better than previous models developed by Heald *et al.* (2000), who used the SNN learning paradigm.

Overall results of the sensitivity analysis of SNN models (Figures 4.3.1.2, 4.3.2.2, 4.3.3.2 and 4.3.4.2) suggested that PC-1, and therefore, SCS and ERI were the most important input variables for discriminating between the three bacteriological states. The FP (through PC-3), days in milk and sire number had a moderate effect; while, PP (through PC-2) and lactation number had very low impact on the CCR of the SNN models.

To achieve the third objective of this research, SNN models were developed using different proportions of infected to non-infected cases in the training datasets. This was done to observe the effect of not keeping to proportions of infected to non-infected cases as found in real data, which is small with a ratio of 1:12. The SNN models were built using different proportions of infected and non-infected cases in the training data set. The CCR for the NI cases increased from 82% to 98%, as the proportion of infected to non-infected cases increased with ratios of 1:1, 1:2, 1:4 and 1:10, confirming that the CCR for NI cases increased when they were more prevalent in the database. These results also indicate that NI cases may be spread within a larger area in the input space compared to infected cases, as was found by Wang and Samarasinghe (2005).

The CCR for the MNI cases decreased from 86% to 44%, as the proportion of NI cases increased in the database, demonstrating that the NI region can overlap with the MNI region when there is a higher prevalence of NI cases. Finally, the CCR for MJI cases increased marginally from 20% to 40% when the proportion of NI cases increased in the database in the ratios previously stated.

This marginal increase may be due to a better separation of this class as the NI cases increased demarcating major infection region better.

The low CCR for the MJI category may be attributed to the low prevalence of this category in the dataset, rather than the capability of the neural network models. This point is supported by the high accuracy of the models for the minor infected category; especially when infected to non-infected ratios were lower (1:1 and 1:2) most probably due to the higher prevalence of this category in this particular herd.

Overall, CCR of infected cases decreased when the number of NI cases increased in the training dataset which agrees with the previous findings (Lacroix, Salehi, Yang, & Wade, 1997; Yang, Lacroix, & Wade, 1999). The current findings revealed that in classification of mastitis, the CCR of a neural network for NI category increases as the number of records in the training data set increases for that category. With more data available it may also be true for MNI and MJI cases as well. Future research may focus on using larger number of cases of each bacteriological state in the training data set to build more robust models.

5.2 Conclusions

Based on the results of this research it is concluded that milk parameters associated with mastitis can be used to build robust ANN models for detecting NI, MNI and MJI quarters. The incorporation of such models in in-line milking systems may improve the efficiency and efficacy of detecting mastitis causing pathogens in milk before any clinical manifestations occur. This may form a reliable basis for managing and controlling mastitis at the farm level.

The USNN model performed better compared to SNN in detecting the infection status of a quarter. The correct classification rate of the SNN for a particular category decreased as the proportion of cases for that category decreased in the training data set. Due to the low prevalence of MJI cases in the dataset, the results of the models for this category may be inconclusive as more data are needed to develop more robust models.

In terms of variables suitable for detecting the infection status of a quarter, SCS and ERI were by far the most informative. The SCS was good for discriminating the three bacteriological stats, while ERI was a good discriminator for MJI. Further research may focus on studying the inclusion of other milk parameters such as milk amyloid A and milk lactate, which may help optimise the discrimination between the three bacteriological states.

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